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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 04 September 1997 (04.09.97)	
International application No.: PCT/AU97/00124	Applicant's or agent's file reference:
International filing date: 28 February 1997 (28.02.97)	Priority date: 01 March 1996 (01.03.96)
Applicant: BRUGLIERA, Filippa et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

14 July 1997 (14.07.97)

[ ] in a notice effecting later election filed with the International Bureau on:

_____

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PN8386/EJH/AF	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. **PCT/AU 97/00124**	International filing date *(day/month/year)* 28 February 1997	(Earliest) Priority Date *(day/month/year)* 1 March 1996

Applicant

(1) Florigene Limited
(2) Brugliera F., et al.

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **3** sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I)

2. [ ] **Unity of invention is lacking** (See Box II)

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [X] filed with the international application

   [ ] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed

   [ ] transcribed by this Authority

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure

   [ ] because this figure better characterises the invention

   [X] None of the figures

INTERNATIONAL SEARCH REPO

International Application No.
PCT/AU 97/00124

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/29, 15/63
A01H 5/00, 5/02

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See electronic databases below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See electronic databases below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO, USPM - ss1: flavonoid(3n)monooxygenase# or flavonoid(3n)hydroxylase# or F3()H; ss2: A01H/IC or C12N-009/IC or C12N-015/IC; ss1 and ss2
CHEMICAL ABSTRACTS - flavonoid 3' hydroxylase (clones pcgp772, pcgp602, pcgp619, pcgp635, pcgp854, pcgp773)/cn; flavonoid 3' hydroxylase/cn, flavonoid 3' monooxygenase
GENEBANK, EMBL, SWISS-PROT - Sequence searches

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU A 37413/93 (INTERNATIONAL FLOWER DEVELOPMENTS PTY. LTD.) 14 OCTOBER 1993 C12N 15/53, 15/11, 9/02, C12Q 1/66, A01H 5/00	27,28,30,31,38
A	PHYTOCHEMISTRY, VOL. 35 PAGES 145-150, 1994, SCHWINN KE et al. "Floral flavonoids and the potential for pelargonidin biosynthesis in commercial chrysanthemum cultivars"	1 - 38

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
9 April 1997

Date of mailing of the international search report
22 APR 1997

Name and mailing address of the ISA/AU
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA Facsimile No.: (06) 285 3929

Authorized officer
KAREN AYERS
Telephone No.: (06) 283 2082

Form PCT/ISA/210 (second sheet) (July 1992) copcaj

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 97/00124

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
AU, A, 37413/93	WO, A, 93/20206
	EP, A, 640136
	NZ, A, 249808

END OF ANNEX

PATENT COOPERATION TREA

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PN8386/EJH/AF	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 97/00124**	International filing date 28 February 1997	Priority Date 1 March 1996
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[6] C12N 15/29, 15/52, 15/82; A01H 4/00, 5/00, 5/02, 5/08, 5/10, 5/12		
Applicant (1) FLORIGENE LIMITED (2) BRUGLIERA F., ET AL.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **4** sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand 14 July 1997	Date of completion of the report 24 October 1997
Name and mailing address of the IPEA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **KAREN AYERS** Telephone No. (02) 6283 2082

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of ,
pages , filed with the letter of .

[ ] the claims, Nos. , as originally filed,
Nos. , as amended under Article 19,
Nos. , filed with the demand,
Nos. , filed with the letter of ,
Nos. , filed with the letter of .

[ ] the drawings, sheets/fig , as originally filed,
sheets/fig , filed with the demand,
sheets/fig , filed with the letter of ,
sheets/fig , filed with the letter of .

2. The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☒ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-26, 29, 32-37**	**YES**
	Claims	**27, 28, 30, 31, 38**	**NO**
Inventive step (IS)	Claims	**1-26, 29, 32-37**	**YES**
	Claims	**27, 28, 30, 31, 38**	**NO**
Industrial applicability (IA)	Claims	**1-38**	**YES**
	Claims		**NO**

2. Citations and explanations

Citations

D1: AU A 37413/93 (International Flower Developments Pty. Ltd.) 14 October 1993 (14.10.93) C12N 15/53, 15/11, 9/02, C12Q 1/66, A01H 5/00

D2: Phytochemistry volume 35, pages 145-150 (1994) by Schwinn KE et al. "Floral flavonoids and the potential for pelargonidin biosynthesis in commercial chrysanthemum cultivars".

D1 is considered to be the closest related art and discloses nucleotide and amino acid sequences encoding a flavonoid-3'-hydroxylase. Claims 1 to 26, 29 and 32 to 37 are restricted to specific sequences encoding flavonoid-3'-hydroxylase which are exemplified in the description as SEQ. ID. Nos: 1-26. However, none of these sequences are disclosed in D1, and therefore claims 1 to 26, 29 and 32 to 37 are novel and inventive in light of D1.

Claims 27, 28, 30, 31 and 38 are not limited to specific sequences exemplified in the description, so are not considered novel or inventive when compared to D1 which discloses a flavonoid-3'-hydroxylase.

D2 defines general information on the role of flavonoid-3'-hydroxylase in flavonoid production and pigmentation of chrysanthemums. No nucleotide or amino acid sequences have been identified and therefore claims 1 to 32 are novel and inventive in light of this document.

Claims 1 to 38 possess industrial applicability for the manipulation of flower colour in the commercial flower industry.

142,108

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

International Patent Classification 6 :		(11) International Publication Number: WO 97/32023
C12N 15/29, 15/63, A01H 5/00, 5/02	A1	(43) International Publication Date: 4 September 1997 (04.09.97)

(21) International Application Number: PCT/AU97/00124

(22) International Filing Date: 28 February 1997 (28.02.97)

(30) Priority Data:
PN 8386 1 March 1996 (01.03.96) AU

(71) Applicant *(for all designated States except US)*: FLORIGENE LIMITED [AU/AU]; 16 Gipps Street, Collingwood, VIC 3066 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BRUGLIERA, Filippa [AU/AU]; 11 Kalimna Street, Preston, VIC 3072 (AU). HOLTON, Timothy, Albert [AU/AU]; Unit 1, 8 May Street, Elwood, VIC 3184 (AU). MICHAEL, Michael, Zenon [AU/AU]; 15 Goodwin Street, West Preston, VIC 3072 (AU).

(74) Agents: HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: GENETIC SEQUENCES ENCODING FLAVONOID PATHWAY ENZYMES AND USES THEREFOR

(57) Abstract

The present invention relates generally to genetic sequences encoding flavonoid pathway metabolising enzymes and more particularly to flavonoid 3'-hydroxylase (hereinafter referred to as "F3'H") or derivatives thereof and their use in the manipulation of pigmentation in plants and other organisms.

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GENETIC SEQUENCES ENCODING FLAVONOID PATHWAY ENZYMES AND USES THEREFOR

The present invention relates generally to genetic sequences encoding flavonoid pathway metabolising enzymes and more particularly to flavonoid 3'-hydroxylase (hereinafter referred to as "F3'H") or derivatives thereof and their use in the manipulation of pigmentation in plants and other organisms.

Bibliographic details of the publications referred to by the author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs) for the nucleotide and amino acid sequences referred to in the specification and claims are defined following the bibliography. A summary of the SEQ ID NOs, and the sequences to which they relate, is provided prior to the Examples.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The rapidly developing sophistication of recombinant DNA technology is greatly facilitating research and development in a range of biotechnology related industries. The horticultural industry has become a recent beneficiary of this technology which has contributed to developments in disease resistance in plants and flowers exhibiting delayed senescence after cutting. Some attention has also been directed to manipulating flower colour.

The flower industry strives to develop new and different varieties of flowering plants. An effective way to create such novel varieties is through the manipulation of flower colour. Classical breeding techniques have been used with some success to produce a wide range of colours for most of the commercial varieties of flowers. This approach has been limited, however, by the constraints of a particular species' gene pool and for this reason it is rare for

a single species to have a full spectrum of coloured varieties. In addition, traditional breeding techniques lack precision. The aesthetic appeal of the flower is a combination of many factors such as form, scent and colour; modification of one character through hybridization can often be at the expense of an equally valuable feature. The ability to genetically engineer precise colour changes in cutflower and ornamental species would offer significant commercial opportunities in an industry which has rapid product turnover and where novelty is an important market characteristic.

Flower colour is predominantly due to two types of pigment: flavonoids and carotenoids. Flavonoids contribute to a range of colours from yellow to red to blue. Carotenoids impart an orange or yellow tinge and are commonly the major pigment in yellow or orange flowers. The flavonoid molecules which make the major contribution to flower colour are the anthocyanins which are glycosylated derivatives of cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin, and are localised in the vacuole. The different anthocyanins can produce marked differences in colour. Flower colour is also influenced by co-pigmentation with colourless flavonoids, metal complexation, glycosylation, acylation and vacuolar pH (Forkmann, 1991).

The biosynthetic pathway for the flavonoid pigments (hereinafter referred to as the "flavonoid pathway") is well established and is shown in Figures 1a and 1b (Ebel and Hahlbrock, 1988; Hahlbrock and Grisebach, 1979; Wiering and De Vlaming, 1984; Schram *et al.*, 1984; Stafford, 1990; Van Tunen and Mol, 1990; Dooner *et al*, 1991; Martin and Gerats, 1993; Holton and Cornish, 1995). The first committed step in the pathway involves the condensation of three molecules of malonyl-CoA with one molecule of *p*-coumaroyl-CoA. This reaction is catalysed by the enzyme chalcone synthase (CHS). The product of this reaction, 2′,4,4′,6′, tetrahydroxy-chalcone, is normally rapidly isomerized to produce naringenin by the enzyme chalcone flavanone isomerase (CHI). Naringenin is subsequently hydroxylated at the 3 position of the central ring by flavanone 3-hydroxylase (F3H) to produce dihydrokaempferol (DHK).

The pattern of hydroxylation of the B-ring of DHK plays a key role in determining petal colour. The B-ring can be hydroxylated at either the 3′, or both the 3′ and 5′ positions, to produce dihydroquercetin (DHQ) and dihydromyricetin (DHM), respectively. Two key enzymes involved in this pathway are flavonoid 3′-hydroxylase and flavonoid 3′,5′-hydroxylase, both of the cytochrome P450 class. Cytochrome P450 enzymes are widespread in nature and genes have been isolated and sequenced from vertebrates, insects, yeasts, fungi, bacteria and plants.

Flavonoid 3′-hydroxylase acts on DHK to produce DHQ and on naringenin to produce eriodictyol. Reduction and glycosylation of DHQ produces the cyanidin-glycoside and peonidin-glycoside pigments which, in many plant species (for example rose, carnation and chrysanthemum), contribute to red and pink flower colour. The synthesis of these anthocyanins can also result in other flower colours. For example, blue cornflowers contain cyanin. The ability to control flavonoid 3′-hydroxylase activity, or other enzymes involved in the flavonoid pathway, in flowering plants would provide a means to manipulate petal colour. Different coloured versions of a single cultivar could thereby be generated and in some instances a single species would be able to produce a broader spectrum of colours.

A nucleotide sequence (referred to herein as SEQ ID NO:26) encoding a petunia flavonoid 3′-hydroxylase has been cloned (see International Patent Application No. PCT/AU93/00127 [WO 93/20206]). However, this sequence was inefficient in its ability to modulate the production of 3′-hydroxylated anthocyanins in plants. There is a need, therefore, to identify further genetic sequences encoding flavonoid 3′-hydroxylases which efficiently modulate the hydroxylation of flavonoid compounds in plants. More particularly, there is a need to identify further genetic sequences encoding flavonoid 3′-hydroxylases which efficiently modulate the production of 3′-hydroxylated anthocyanins in plants.

In accordance with the present invention, genetic sequences encoding flavonoid 3′-hydroxylase have been identified and cloned. The recombinant genetic sequences of the present invention permit the modulation of expression of genes encoding this enzyme by, for

example, *de novo* expression, over-expression, suppression, antisense inhibition and ribozyme activity. The ability to control flavonoid 3'-hydroxylase synthesis in plants permits modulation of the composition of individual anthocyanins as well as alteration of relative levels of flavonols and anthocyanins, thereby enabling the manipulation of tissue colour, such as petals, leaves, seeds and fruit. The present invention is hereinafter described in relation to the manipulation of flower colour but this is done with the understanding that it extends to manipulation of other plant tissues, such as leaves, seeds and fruit.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding a flavonoid 3'-hydroxylase or a derivative thereof wherein said flavonoid 3'-hydroxylase or its derivative is capable of more efficient modulation of hydroxylation of flavonoid compounds in plants than is a flavonoid 3'-hydroxylase encoded by the nucleotide sequence set forth in SEQ ID NO:26.

Efficiency as used herein relates to the capability of the flavonoid 3'-hydroxylase enzyme to hydroxylate flavonoid compounds in a plant cell. This provides the plant with additional substrates for other enzymes of the flavonoid pathway able to further modify this molecule, via, for example, glycosylation, acylation and rhamnosylation, to produce various anthocyanins which contribute to the production of a range of colours. The modulation of 3'-hydroxylated anthocyanins is thereby permitted. Efficiency is conveniently assessed by one or more parameters selected from: extent of transcription, as determined by the amount of mRNA produced; extend of hydroxylation of naringenin and/or DHK; extent of translation of mRNA, as determined by the amount of translation product produced; extent of production of anthocyanin derivatives of DHQ or DHM; the extent of effect on tissue colour, such as flowers, seeds, leaves or fruits.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides which maps to the genetic locus designated Ht1 or Ht2 in petunia, or to equivalent such loci in other flowering plant species, and wherein said isolated nucleic acid molecule encodes, or is complementary to a sequence which encodes,

a flavonoid 3'-hydroxylase.

A further aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides which corresponds to the genetic locus designated Ht1 or Ht2 in petunia, or to loci in other flowering plant species which contain sequences which control production of 3'-hydroxylated flavonoids, and wherein said isolated nucleic acid molecule encodes a flavonoid 3'-hydroxylase or a derivative thereof which is capable of more efficient conversion of DHK to DHQ in plants than is the flavonoid 3'-hydroxylase set forth in SEQ ID NO:26.

In accordance with the above aspects of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:1 under low stringency conditions.

In a related embodiment, there is provided a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions.

In another related embodiment, the present invention is directed to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:5 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:5 under low stringency conditions.

Yet another related embodiment provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:7 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions.

Still yet a further embodiment of the present invention relates to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:9 or having at least about 60% similarity to the coding region thereof or capable of hybridising to the sequence set forth in SEQ ID NO:9 under low stringency conditions.

In another further embodiment, there is provided a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:14 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:14 under low stringency conditions.

In yet another further embodiment, the present invention is directed to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:16 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:16 under low stringency conditions.

Still yet another further embodiment provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:18 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:18 under low stringency conditions.

Moreover, yet a further embodiment of the present invention relates to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:20 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:20 under low stringency conditions.

Still yet another further embodiment is directed to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:22 or having at least about 60% similarity thereto or capable of hybridising to the

sequence set forth in SEQ ID NO:22 under low stringency conditions.

In still yet another further embodiment, the present invention provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:24 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:24 under low stringency conditions.

In a particularly preferred embodiment there is provided an isolated nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:1 under low stringency conditions, wherein said nucleotide sequence maps to the genetic locus designated Ht1 or Ht2 in petunia, or to equivalent such loci in other flowering plant species, and wherein said isolated nucleic acid molecule encodes, or is complementary to a sequence which encodes, a flavonoid 3′-hydroxylase.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% to at least about 15% formamide and from at least about 1M to at least about 2M salt for hybridization, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% to at least about 30% formamide and from at least about 0.5M to at least about 0.9M salt for hybridization, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% to at least about 50% formamide and from at least about 0.01M to at least about 0.15M salt for hybridization, and at least about 0.01M to at least about 0.15M salt for washing conditions. Hybridization may be carried out at different temperatures and, where this occurs, other conditions may be adjusted accordingly.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid

sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least about 50% similarity thereto.

In a related embodiment, there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least about 50% similarity thereto.

A further related embodiment of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity thereto.

Still another related embodiment provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

Yet still another related embodiment relates to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:10 or SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13 or an amino acid sequence having at least about 50% similarity thereto.

In another further embodiment, there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:15 or an amino acid sequence having at least about 50% similarity thereto.

In yet another further embodiment, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an

amino acid sequence substantially as set forth in SEQ ID NO:17 or an amino acid sequence having at least about 50% similarity thereto.

Still yet another further embodiment provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:19 or an amino acid sequence having at least about 50% similarity thereto.

Moreover, yet a further embodiment of the present invention relates to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:21 or an amino acid sequence having at least about 50% similarity thereto.

Still yet another further embodiment is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:23 or an amino acid sequence having at least about 50% similarity thereto.

In still yet another further embodiment, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:25 or an amino acid sequence having at least about 50% similarity thereto.

In a particularly preferred embodiment there is provided an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least about 50% similarity thereto, wherein said sequence of nucleotides maps to the genetic locus designated Ht1 or Ht2 in petunia, or to equivalent such loci in other flowering plant species, and wherein said isolated nucleic acid molecule encodes, or is complementary to a sequence which encodes, a flavonoid 3′-hydroxylase or a derivative

therof.

The term "similarity" as used herein includes exact identity between compared sequences, at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels.

The nucleic acid molecule defined by SEQ ID NO:1 encodes a flavonoid 3'-hydroxylase from petunia. Examples of other suitable F3'H genes are from carnation (SEQ ID NO:3), snapdragon (SEQ ID NO:5), arabidopsis (SEQ ID NO:7), arabidopsis genomic DNA clone (SEQ ID NO: 9), rose (SEQ ID NO:14), chrysanthemum (SEQ ID NO:16), torenia (SEQ ID NO:18), Japanese morning glory (SEQ ID NO:20), gentian (SEQ ID NO:22) and lisianthus (SEQ ID NO:24). Although the present invention is particularly exemplified by the aforementioned F3'H genes, the subject invention extends to F3'H genes from any species of plant provided that the F3'H gene has at least about 60% similarity at the nucleotide level to a nucleic acid molecule selected from SEQ ID NO:1 or 3 or 5 or 7 or 14 or 16 or 18 or 20 or 22 or 24, or at least about 50% similarity at the amino acid level to an amino acid molecule selected from SEQ ID NO: 2 or 4 or 6 or 8 or 10, 11, 12, 13 or 15 or 17 or 19 or 21 or 23 or 25. The subject invention further extends to F3'H genes from any species of plant provided that the F3'H gene has at least about 60% similarity at the nucleotide level to the coding region of SEQ ID NO:9.

The nucleic acid molecules of the present invention are generally genetic sequences in a non-naturally-occurring condition. Generally, this means isolated away from its natural state or synthesized or derived in a non-naturally-occurring environment. More specifically, it includes nucleic acid molecules formed or maintained *in vitro,* including genomic DNA fragments, recombinant or synthetic molecules and nucleic acids in combination with

heterologous nucleic acids. It also extends to the genomic DNA or cDNA or part thereof encoding F3'H or part thereof in reverse orientation relative to its or another promoter. It further extends to naturally-occurring sequences following at least a partial purification relative to other nucleic acid sequences.

The term "nucleic acid molecule" includes a nucleic acid isolate and a genetic sequence and is used herein in its most general sense and encompasses any contiguous series of nucleotide bases specifying directly, or *via* a complementary series of bases, a sequence of amino acids in a F3'H. Such a sequence of amino acids may constitute a full-length F3'H or an active truncated form thereof or may correspond to a particular region such as an N-terminal, C-terminal or internal portion of the enzyme. The nucleic acid molecules contemplated herein also encompass oligonucleotides useful as genetic probes or as "antisense" molecules capable of regulating expression of the corresponding gene in a plant. An "antisense molecule" as used herein may also encompass a gene construct comprising the structural genomic or cDNA gene or part thereof in reverse orientation relative to its own or another promoter. Accordingly, the nucleic acid molecules of the present invention may be suitable for use as cosuppression molecules, ribozyme molecules, sense molecules and antisense molecules to modulate levels of 3'-hydroxylated anthocyanins.

In one embodiment, the nucleic acid molecule encoding F3'H or various derivatives thereof is used to reduce the activity of an endogenous F3'H, or alternatively the nucleic acid molecule encoding this enzyme or various derivatives thereof is used in the antisense orientation to reduce activity of the F3'H. Although not wishing to limit the present invention to any one theory, it is possible that the introduction of the nucleic acid molecule into a cell results in this outcome either by decreasing transcription of the homologous endogenous gene or by increasing turnover of the corresponding mRNA. This may be achieved using gene constructs containing F3'H nucleic acid molecules or various derivatives thereof in either the sense or the antisense orientation. In a further alternative, ribozymes could be used to inactivate target nucleic acid molecules. Alternatively, the nucleic acid molecule encodes a functional F3'H and this is used to elevate levels of this enzyme in plants.

Reference herein to the altering of flavonoid F3'H activity relates to an elevation or reduction in activity of up to 30% or more preferably of 30-50%, or even more preferably 50-75% or still more preferably 75% or greater above or below the normal endogenous or existing levels of activity. The level of activity can be readily assayed using a modified version of the method described by Stotz and Forkmann (1982) (see Example 7) or by assaying for the amount of F3'H product such as quercetin, cyanidin or peonidin as set forth in Example 5.

The present invention further extends to nucleic acid molecules in the form of oligonucleotide primers or probes capable of hybridizing to a portion of the nucleic acid molecules contemplated above, and in particular those selected from the nucleic acid molecules set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 14, 16, 18, 20, 22 or 24 under high, preferably under medium and most preferably under low stringency conditions. Preferably the portion corresponds to the 5' or the 3' end of the F3'H gene. For convenience the 5' end is considered herein to define a region substantially between the 5' end of the primary transcript to a centre portion of the gene, and the 3' end is considered herein to define a region substantially between the centre portion of the gene and the 3' end of the primary transcript. It is clear, therefore, that oligonucleotides or probes may hybridize to the 5' end or the 3' end or to a region common to both the 5' and the 3' ends.

The nucleic acid molecule or its complementary form may encode the full-length enzyme or a part or derivative thereof. By "derivative" is meant any single or multiple amino acid substitutions, deletions, and/or additions relative to the naturally-occurring enzyme and includes parts, fragments, portions, fusion molecules, homologues and analogues. In this regard, the nucleic acid includes the naturally-occurring nucleotide sequence encoding F3'H or may contain single or multiple nucleotide substitutions, deletions and/or additions to said naturally-occurring sequence. A fusion molecule may be a fusion between nucleotide sequences encoding two or more F3'Hs, or a fusion between a nucleotide sequence encoding an F3'H and a nucleotide sequence encoding any other proteinaceous molecule. Fusion molecules are useful in altering substrate specificity.

A derivative of the nucleic acid molecule or its complementary form, or of a F3'H, of the present invention may also include a "part", whether active or inactive. An active or functional nucleic acid molecule is one which encodes an enzyme with F3'H activity. An active or functional molecule further encompasses a partially-active molecule; for example, an F3'H with reduced substrate specificity would be regarded as partially active. A derivative of a nucleic acid molecule may be useful as an oligonucleotide probe, as a primer for polymerase chain reactions or in various mutagenic techniques, for the generation of antisense molecules or in the construction of ribozymes. They may also be useful in developing co-suppression constructs. The nucleic acid molecule according to this aspect of the present invention may or may not encode a functional F3'H. A "part" may be derived from the 5' end or the 3' end or a region common to both the 5' and the 3' ends of the nucleic acid molecule.

Amino acid insertional derivatives of the F3'H of the present invention include amino and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Typical substitutions are those made in accordance with Table 1 below.

TABLE 1

Suitable residues for amino acid substitutions

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Where the F3'H is derivatised by amino acid substitution, the amino acids are generally replaced by other amino acids having like properties, such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains and the like. Amino acid substitutions are typically of single residues. Amino acid insertions will usually be in the order of about 1-10 amino acid residues and deletions will range from about 1-20 residues. Preferably, deletions or insertions are made in adjacent pairs, i.e. a deletion of two residues or insertion of two residues.

The amino acid variants referred to above may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis (Merrifield, 1964) and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known or partially known sequence are well known and include, for example, M13 mutagenesis. The manipulation of DNA sequence to produce variant proteins which manifest as substitutional, insertional or deletional variants are conveniently described, for example, in Sambrook *et al.* (1989).

Other examples of recombinant or synthetic mutants and derivatives of the F3'H of the present invention include single or multiple substitutions, deletions and/or additions of any molecule associated with the enzyme such as carbohydrates, lipids and/or proteins or polypeptides.

The terms "analogues" and "derivatives" also extend to any chemical equivalents of the F3'H, whether functional or not, and also to any amino acid derivative described above. Where the "analogues" and "derivatives" of this aspect of the present invention are non-functional, they may act as agonists or antagonists of F3'H activity. For convenience, reference to "F3'H" herein includes reference to any derivatives, including parts, mutants, fragments, homologues or analogues thereof.

The present invention is exemplified using nucleic acid sequences derived from petunia, carnation, rose, snapdragon, arabidopsis, chrysanthemum, lisianthus, torenia, morning glory and gentian, since these represent the most convenient and preferred sources of material to date. However, one skilled in the art will immediately appreciate that similar sequences can be isolated from any number of sources such as other plants or certain microorganisms. Examples of other plants include, but are not limited to, maize, tobacco, cornflower, pelargonium, apple, gerbera and african violet. All such nucleic acid sequences encoding directly or indirectly a flavonoid pathway enzyme and in particular F3'H, regardless of their source, are encompassed by the present invention.

The nucleic acid molecules contemplated herein may exist in either orientation alone or in combination with a vector molecule, for example an expression-vector. The term vector molecule is used in its broadest sense to include any intermediate vehicle for the nucleic acid molecule, capable of facilitating transfer of the nucleic acid into the plant cell and/or facilitating integration into the plant genome. An intermediate vehicle may, for example, be adapted for use in electroporation, microprojectile bombardment, *Agrobacterium*-mediated transfer or insertion via DNA or RNA viruses. The intermediate vehicle and/or the nucleic acid molecule contained therein may or may not need to be stably integrated into the plant genome. Such vector molecules may also replicate and/or express in prokaryotic cells. Preferably, the vector molecules or parts thereof are capable of integration into the plant genome. The nucleic acid molecule may additionally contain a promoter sequence capable of directing expression of the nucleic acid molecule in a plant cell. The nucleic acid molecule and promoter may also be introduced into the cell by any number of means such as those described above.

In accordance with the present invention, a nucleic acid molecule encoding a F3'H or a derivative or part thereof may be introduced into a plant in either orientation to allow, permit or otherwise facilitate manipulation of levels of production of mRNA in the cytoplasm and/or production of enzyme from the mRNA, thereby providing a means either to convert DHK and/or other suitable substrates, if synthesised in the plant cell, ultimately into anthocyanin derivatives of anthocyanidins such as cyanidin and/or peonidin, or alternatively to inhibit such conversion of metabolites by reducing or eliminating endogenous or existing F3'H activity. The production of mRNA in the cytoplasm and/or production of enzyme from the mRNA, is referred to herein as "expression". The production of anthocyanins contributes to the production of a red or blue flower colour. Expression of the nucleic acid molecule in either orientation in the plant may be constitutive, inducible or developmental, and may also be tissue-specific.

According to this aspect of the present invention there is provided a method for producing a transgenic plant capable of synthesizing F3'H or functional derivatives thereof, said method

comprising stably transforming a cell of a suitable plant with a nucleic acid molecule which comprises a sequence of nucleotides encoding said F3'H, under conditions permitting the eventual expression of said nucleic acid molecule, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid molecule. The transgenic plant may thereby produce elevated levels of F3'H activity relative to the amount expressed in a comparable non-transgenic plant.

Another aspect of the present invention contemplates a method for producing a transgenic plant with reduced endogenous or existing F3'H activity, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence encoding F3'H, regenerating a transgenic plant from the cell and where necessary growing said transgenic plant under conditions sufficient to permit the expression of the nucleic acid molecule.

Yet another aspect of the present invention contemplates a method for producing a genetically modified plant with reduced endogenous or existing F3'H activity, said method comprising altering the F3'H gene through modification of the endogenous sequences via homologous recombination from an appropriately altered F3'H gene or derivative or part thereof introduced into the plant cell, and regenerating the genetically modified plant from the cell.

In accordance with these aspects of the present invention the preferred nucleic acid molecules are substantially as set forth in SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22, 24, or the coding region of 9, or have at least about 60% similarity thereto, or are capable of hybridising thereto under low stringency conditions.

In a preferred embodiment, the present invention contemplates a method for producing a transgenic flowering plant exhibiting altered flower colour, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule of the present invention, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid molecule into the F3'H

enzyme. Alternatively, said method may comprise stably transforming a cell of a suitable plant with a nucleic acid molecule of the present invention or its complementary sequence, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to alter the level of activity of the endogenous or existing F3'H. Preferably, the altered level would be less than the endogenous or existing level of F3'H activity in a comparable non-transgenic plant.

In a related embodiment, the present invention contemplates a method for producing a flowering plant exhibiting altered flower colour, said method comprising alteration of the F3'H gene through modification of the endogenous sequences *via* homologous recombination from an appropriately altered F3'H gene or derivative thereof introduced into the plant cell and regenerating the genetically modified plant from the cell.

The nucleic acid molecules of the present invention may or may not be developmentally regulated. Preferably, the modulation of levels of 3'-hydroxylated anthocyanins leads to altered flower colour which includes the production of red flowers or other colour shades depending on the physiological conditions of the recipient plant. By "recipient plant" is meant a plant capable of producing a substrate for the F3'H enzyme, or producing the F3'H enzyme itself, and possessing the appropriate physiological properties and genotype required for the development of the colour desired. This may include but is not limited to petunia, carnation, chrysanthemum, rose, snapdragon, tobacco, cornflower, pelargonium, lisianthus, gerbera, apple, iris, lily, african violet, gentian, torenia and Japanese morning glory.

Accordingly, the present invention extends to a method for producing a transgenic plant capable of modulating levels of 3'-hydroxylated anthocyanins, said method comprising stably transforming a cell or group of cells of a suitable plant with a nucleic acid molecule comprising a sequence of nucleotides encoding, or complementary to a sequence encoding, F3'H or a derivative thereof, and regenerating a transgenic plant from said cell or cells.

One skilled in the art will immediately recognise the variations applicable to the methods of

the present invention, such as increasing or decreasing the level of enzyme activity of the enzyme naturally present in a target plant leading to differing shades of colours.

The present invention, therefore, extends to all transgenic plants containing all or part of the nucleic acid module of the present invention and/or any homologues or related forms thereof or antisense forms of any of these and in particular those transgenic plants which exhibit altered flower colour. The transgenic plants may contain an introduced nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding F3'H. Generally, the nucleic acid would be stably introduced into the plant genome, although the present invention also extends to the introduction of the F3'H nucleotide sequence within an autonomously-replicating nucleic acid sequence such as a DNA or RNA virus capable of replicating within the plant cell. The invention also extends to seeds from such transgenic plants. Such seeds, especially if coloured, will be useful as proprietary tags for plants.

A further aspect of the present invention is directed to recombinant forms of F3'H. The recombinant forms of the enzymes will provide a source of material for research to develop, for example, more active enzymes and may be useful in developing *in vitro* systems for production of coloured compounds.

Still a further aspect of the present invention contemplates the use of the genetic sequences described herein in the manufacture of a genetic construct capable of use in modulating levels of 3'-hydroxylated anthocyanins in a plant or cells of a plant.

Yet a further aspect of the present invention provides flowers and in particular cut flowers, from the transgenic plants herein described, exhibiting altered flower colour.

Another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding, a F3'H or a derivative thereof wherein said nucleic acid molecule is capable of being expressed in a plant cell. The term "expressed" is equivalent to the term "expression" as defined above.

The nucleic acid molecules according to this and other aspects of the invention allow, permit or otherwise facilitate increased efficiency in modulation of 3'-hydroxylated anthocyanins relative to the efficency of the pCGP619 cDNA insert contained in plasmid pCGP809, disclosed in International Patent Application No. PCT/AU93/00127 [WO 93/20206]. The term "plant cell" includes a single plant cell or a group of plant cells such as in a callus, plantlet or plant or parts thereof including flowers and seeds.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence of nucleotides encoding a F3'H, wherein the translation of the said nucleic acid molecule comprises the amino acid sequence RPPNSGA. Preferably, the translation of the said nucleic acid molecule comprises the amino acid sequence RPPNSGAXHXAYNYXDL and still more preferably the translation of the said nucleic acid molecule comprises the amino acid sequence RPPNSGAXHXAYNYXDL$[X]_n$GGEK, where X represents any amino acid and $[X]_n$ represents an amino acid sequence of from 0 to 500 amino acids.

The present invention is further described by reference to the following non-limiting Figures and Examples.

In the Figures:

Figures 1a and 1b are schematic representations of the flavonoid biosynthesis pathways in *P. hybrida* flowers showing the enzymes and genetic loci involved in the conversions. Enzymes involved in the pathway have been indicated as follows: PAL = phenylalanine ammonia-lyase; C4H = cinnamate 4-hydroxylase; 4CL = 4-coumarate: CoA ligase; CHS= chalcone synthase; CHI= chalcone isomerase; F3H= flavanone 3-hydroxylase; F3'H= flavonoid 3'-hydroxylase; F3'5'H= flavonoid 3'5' hydroxylase; FLS= flavonol synthase; DFR= dihydroflavonol-4-reductase; ANS= anthocyanin synthase; 3GT= UDP-glucose: anthocyanin-3-glucoside; 3RT= UDP-rhamnose: anthocyanidin-3-glucoside rhamnosyltransferase; ACT= anthocyanidin-3-rutinoside acyltransferase; 5GT= UDP-

glucose: anthocyanin 5- glucosyltransferase; 3' OMT = anthocyanin *O*-methyltransferase; 3', 5' OMT=anthocyanin 3', 5' *O*-methyltransferase. Three flavonoids in the pathway are indicated as: P-3-G= pelargonidin-3-glucoside; DHM=dihydomyricetin; DHQ=dihydroquercetin. The flavonol, myricetin, is only produced at low levels and the anthocyanin, pelargonidin, is rarely produced in *P. hybrida*.

Figure 2 is a diagrammatic representation of the plasmid pCGP161 containing a cDNA clone (F1) representing the cinnamate-4-hydroxylase from *P. hybrida*. ^{32}P-labelled fragments of the 0.7 kb EcoRI/XhoI fragment were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp = the ampicillin resistance gene; ori = origin of replication; T3 = recognition sequence for T3 RNA polymerase; T7 = recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

Figure 3 is a diagrammatic representation of the plasmid pCGP602 containing a cDNA clone (617) representing a flavonoid 3'5' hydroxylase (Hf1) from *P. hybrida*. ^{32}P-labelled fragments of the 1.6 kb BspHI/FspI fragment containing the Hf1 coding region were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp = the ampicillin resistance gene; ori = origin of replication; T3 = recognition sequence for T3 RNA polymerase; T7 = recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

Figure 4 is a diagrammatic representation of the plasmid pCGP175 containing a cDNA clone (H2) representing a flavonoid 3'5' hydroxylase (Hf2) from *P. hybrida*. ^{32}P-labelled fragments of the 1.3 kb EcoRI/XhoI and 0.5 kb XhoI fragments which together contain the Hf2 coding region were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp = the ampicillin resistance gene; ori = origin of replication; T3 = recognition sequence for T3 RNA polymerase; T7 = recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

Figure 5 is a diagrammatic representation of the plasmid pCGP619 containing the 651 cDNA clone representing a cytochrome P450 from *P. hybrida*. ^{32}P-labelled fragments of the 1.8 kb EcoRI/XhoI fragment were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp = the ampicillin resistance gene; ori = origin of replication; T3 = recognition sequence for T3 RNA polymerase; T7 = recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

Figure 6 is a representation of an autoradiograph of an RNA blot probed with ^{32}P-labelled fragments of the OGR-38 cDNA clone contained in pCGP1805 (see Example 6). Each lane contained a 20 μg sample of total RNA isolated from the flowers or leaves of plants of a V23 (ht1/ht1) x VR (Ht1/ht1) backcross population. A 1.8 kb transcript was detected in the VR-like (Ht1/ht1) flowers that contained high levels of quercetin (Q+)(lanes 9 - 14). The same size transcript was detected at much lower levels in the V23-like (ht1/ht1) flowers that contained little or no quercetin (Q-) (lanes 3-8). A reduced level of transcript was also detected in VR leaves (lane 1) and V23 petals (lane 2). This is described in Example 5.

Figure 7 is a diagrammatic representation of the yeast expression plasmid pCGP1646 (see Example 7). The OGR-38 cDNA insert from pCGP1805 was cloned in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter (PGAP) in the expression vector pYE22m. TRP1 = Trp1 gene, IR1 = inverted repeat of 2 μm plasmid, TGAP = terminator sequence from the yeast glyceraldehyde-3-phosphate dehydrogenase gene. Restriction enzyme sites are also marked.

Figure 8 is a diagrammatic representation of the binary plasmid pCGP1867 (described in Example 8). The Ht1 cDNA insert (OGR-38) from pCGP1805 was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB = left border; RB = right border; Gm = the gentamycin resistance gene; 35S = the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII = the neomycin phosphotransferase II gene; tml3' = the terminator region from the tml gene of *Agrobacterium*; mas3' = the terminator region from the mannopine synthase gene of

Agrobacterium; ori pRi = a broad host range origin of replication from an *Agrobacterium rhizogenes* plasmid; oriColE1 = a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

Figure 9 is a diagrammatic representation of the binary plasmid pCGP1810, preparation of which is described in Example 13. The KC-1 cDNA insert from pCGP1807 (see Example 12) was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB = left border; RB = right border; Gm = the gentamycin resistance gene; 35S = the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII = the neomycin phosphotransferase II gene; tml3' = the terminator region from the tml gene of *Agrobacterium*; mas3' = the terminator region from the mannopine synthase gene of *Agrobacterium*; ori pRi = a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1 = a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

Figure 10 is a diagrammatic representation of the binary plasmid pCGP1813, construction of which is described in Example 14. The KC-1 cDNA insert from pCGP1807 (see Example 12) was cloned in a "sense" orientation between the mac promoter and mas terminator. The Mac: KC-1: mas expression cassette was subsequently cloned into the binary vector pWTT2132. Abbreviations are as follows: Tet= the tetracycline resistance gene; LB= left border; RB=right border, surB=the coding region and terminator sequence from the acetolactate synthase gene; 35S= the promoter region from the cauliflower mosaic virus 35S gene, mas3'=the terminator region from the mannopine synthase gene of *Agrobacterium;* pVS1 = a broad host range origin of replication from a plasmid from *Pseodomonas aeruginosa,* pACYCori= modified replicon from pACYC184 from *E. coli.* Restriction enzyme sites are also marked.

Figure 11 is a representation of an autoradiograph of a Southern blot probed with ^{32}P-labelled fragments of the Am3Ga differential display PCR fragment (as described in Example 16). Each lane contained a 10 μg sample of EcoRV-digested genomic DNA isolated from N8

(Eos$^+$), K16 (eos$^-$) or plants of an K16 x N8 F_2 population. Hybridizing bands were detected in the genomic DNA from cyanidin-producing plants (indicated with "+") (Lanes 1, 3, 4, 5, 6, 7, 9, 10, 12 and 15). No specific hybridization was observed in the genomic DNA samples from non-cyanidin-producing plants (indicated with "-") (Lanes 2, 8, 11, 13 and 14).

Figure 12 is a representation of an autoradiograph of an RNA blot probed with ^{32}P-labelled fragments of the Am3Ga differential display PCR fragment. Each lane contained a 10 μg sample of total RNA isolated from the flowers or leaves of plants of an N8 (Eos$^+$) x K16 (eos$^-$) F_2 population. A 1.8 kb transcript was detected in the K16 x N8 F_2 flowers that produced cyanidin (cyanidin +) (plants #1, #3, #4, #5 and #8). No transcript was detected in the K16 x N8 F_2 flowers that did not produce cyanidin (cyanidin -) (plants #6, #11, #12) or in a leaf sample (#13L) from an K16 x N8 F_2 plant that produced cyanidin in the flowers. Details are provided in Example 17.

Figure 13 is a diagrammatic representation of the binary plasmid pCGP250, construction of which is described in Example 20. The sdF3'H cDNA insert, containing the nucleotides 1 through to 1711 (SEQ ID NO:5) from pCGP246 (see Example 18), was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB = left border; RB = right border; Gm = the gentamycin resistance gene; 35S = the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII = the neomycin phosphotransferase II gene; tml3' = the terminator region from the tml gene of *Agrobacterium*; mas3' = the terminator region from the mannopine synthase gene of *Agrobacterium*; ori pRi = a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1 = a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

Figure 14 is a diagrammatic representation of the binary plasmid pCGP231, construction of which is described in Example 20. The sdF3'H cDNA insert, containing the nucleotides 104 through to 1711 (SEQ ID NO:5) from pCGP246, was cloned in a "sense" orientation behind

the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB = left border; RB = right border; Gm = the gentamycin resistance gene; 35S = the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII = the neomycin phosphotransferase II gene; tml3' = the terminator region from the tml gene of *Agrobacterium*; mas3' = the terminator region from the mannopine synthase gene of *Agrobacterium*; ori pRi = a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1 = a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

Figure 15 is a diagrammatic representation of the binary plasmid pBI-Tt7-2. The 6.5 kb EcoRI/SalI Tt7 genomic fragment from E-5 was cloned into EcoRI/SalI-cut pBI101, replacing the resident GUS gene. The orientation of the Tt7 (F3'H) gene as indicated (5' to 3') was determined through DNA sequencing. Abbreviations are as follows: LB = left border; RB = right border; nos 5' = the promoter region from the nopaline synthase gene of *Agrobacterium;* nptII = the coding region of the neomycin phosphotransferase II gene; nos 3' = the terminator region from the nopaline synthase gene of *Agrobacterium;* nptI = the coding region of the neomycin phosphotransferase I gene. Restriction enzyme sites are also marked.

Figure 16 is a diagrammatic representation of the binary plasmid pCGP2166, construction of which is described in Example 26. The rose #34 cDNA insert from pCGP2158 (see Example 25) was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB = left border; RB = right border; Gm = the gentamycin resistance gene; 35S = the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII = the neomycin phosphotransferase II gene; tml3' = the terminator region from the tml gene of *Agrobacterium*; mas3' = the terminator region from the mannopine synthase gene of *Agrobacterium*; ori pRi = a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1 = a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

Figure 17 is a diagrammatic representation of the binary plasmid pCGP2169 construction of which is described in Example 27. The rose #34 cDNA insert from pCGP2158 was cloned in a "sense" orientation between the CaMV35S promoter and the ocs terminator. The 35S: rose #34: ocs expression cassette was subsequently cloned into the binary vector pWTT2132. Abbreviations are as follows: Tet= the tetracycline resistance gene; LB= left border; RB=right border; surB=the boding region and terminator sequence from the acetolactate synthase gene; 35S=the promoter region from the cauliflowe mosaic virus 35S gene, ocs=terminator region from the octopine synthase gene from *Agrobacterium;* pVS1=a broad host range origin of replication from a plasmid from *Pseodomous aeruginosa,* pACYCori=modified replicon from pACYC184 from *E. coli.* Restriction enzyme sites are also marked.

Figure 18 is a diagrammatic representation of the binary plasmid pLN85, construction of which is described in Example 28. The chrysanthemum RM6i cDNA insert from pCHRM1 was cloned in "anti-sense" orientation behind the promoter from the Cauliflower Mosaic Virus 35S gene (35S). Other abbreviations are as follows: LB = left border; RB = right border; ocs3' = the terminator region from the octopine synthase gene of *Agrobacterium*; pnos:nptII:nos 3' = the expression cassette containing the promoter region from the nopaline synthase gene of *Agrobacterium*; the coding region of the neomycin phosphotransferase II gene and the terminator region from the nopaline synthase gene of *Agrobacterium*; oriT = origin of transfer of replication; trfA* = a trans-acting replication function; oriColE1 = a high copy origin of replication from a Colcinin E1 plasmid; Tn7SpR/StR = the spectinomycin and streptomycin resistance genes from transposon Tn7; oriVRK2 = a broad host range origin of replication from plasmid RK2. Restriction enzyme sites are also marked.

Figure 19 is a diagrammatic representation of the yeast expression plasmid pYTHT6, construction of which is described in Example 30. The THT6 cDNA insert from pTHT6 was cloned in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter (PGAP) in the expression vector pYE22m. Abbreviations are as follows: TRP1 = Trp1 gene; IR1 = inverted repeat of 2 μm plasmid; TGAP = the terminator sequence from

the yeast glyceraldehyde-3-phosphate dehydrogenase gene. Restriction enzyme sites are also marked.

Amino acid abbreviations used throughout the specification are shown in Table 2, below.

TABLE 2

Amino acid abbreviations

Amino acid	3-letter	single-letter
L-alanine	Ala	A
L-arginine	Arg	R
L-asparagine	Asn	N
L-aspartic acid	Asp	D
L-cysteine	Cys	C
L-glutamine	Gln	Q
L-glutamic acid	Glu	E
L-glycine	Gly	G
L-histidine	His	H
L-isoleucine	Ile	I
L-leucine	Leu	L
L-lysine	Lys	K
L-methionine	Met	M
L-phenylalanine	Phe	F
L-proline	Pro	P
L-serine	Ser	S
L-threonine	Thr	T
L-tryptophan	Trp	W
L-tyrosine	Tyr	Y
L-valine	Val	V

Table 3 provides a summary of the SEQ ID NO's assigned to the sequences referred to herein:

TABLE 3

Sequence	Species	SEQ ID NO
cDNA insert of pCGP1805	Petunia	SEQ ID NO:1
corresponding amino acid sequence	Petunia	SEQ ID NO:2
cDNA insert of pCGP1807	Carnation	SEQ ID NO:3
corresponding amino acid sequence	Carnation	SEQ ID NO:4
cDNA insert of pCGP246	Snapdragon	SEQ ID NO:5
corresponding amino acid sequence	Snapdragon	SEQ ID NO:6
cDNA partial sequence	Arabidopsis	SEQ ID NO:7
corresponding amino acid sequence	Arabidopsis	SEQ ID NO:8
genomic sequence	Arabidopsis	SEQ ID NO:9
corresponding amino acid sequence for exon I	Arabidopsis	SEQ ID NO:10
corresponding amino acid sequence for exon II	Arabidopsis	SEQ ID NO:11
corresponding amino acid sequence for exon III	Arabidopsis	SEQ ID NO:12
corresponding amino acid sequence for exon IV	Arabidopsis	SEQ ID NO:13
cDNA insert of pCGP2158	Rose	SEQ ID NO:14
corresponding amino acid sequence	Rose	SEQ ID NO:15
cDNA insert of pCHRM1	Chrysanthemum	SEQ ID NO:16
corresponding amino acid sequence	Chrysanthemum	SEQ ID NO:17
THT cDNA sequence	Torenia	SEQ ID NO:18
corresponding amino acid sequence	Torenia	SEQ ID NO:19

MHT 85 cDNA sequence	Jap. Morning Glory	SEQ ID NO:20
corresponding amino acid sequence	Jap. Morning Glory	SEQ ID NO:21
GHT13 cDNA sequence	Gentian	SEQ ID NO:22
corresponding amino acid sequence	Gentian	SEQ ID NO:23
cDNA insert of pL3-6	Lisianthus	SEQ ID NO:24
corresponding amino acid sequence	Lisianthus	SEQ ID NO:25
cDNA sequence from WO 93/20206	Petunia	SEQ ID NO:26
oligonucleotide polyT-anchA		SEQ ID NO:27
oligonucleotide polyT-anchC		SEQ ID NO:28
oligonucleotide polyT-anchG		SEQ ID NO:29
conserved amino acid primer region		SEQ ID NO:30
corresponding oligonucleotide sequence		SEQ ID NO:31
conserved amino acid primer region		SEQ ID NO:32
corresponding oligonucleotide sequence		SEQ ID NO:33
oligonucleotide primer Pet Haem-New		SEQ ID NO:34
conserved amino acid primer region		SEQ ID NO:35
corresponding oligonucleotide sequence		SEQ ID NO:36
oligonucleotide Snapred Race A		SEQ ID NO:37
oligonucleotide Snapred Race C		SEQ ID NO:38
oligonucleotide poly-C Race		SEQ ID NO:39
oligonucleotide primer Pet Haem		SEQ ID NO:40

The disarmed microorganism *Agrobacterium tumefaciens* strain AGL0 separately containing the plasmids pCGP1867, pCGP1810 and pCGP231 were deposited with the Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, New South Wales, 2037, Australia on 23 February, 1996 and were given Accession Numbers 96/10967, 96/10968 and 96/10969, respectively.

ISOLATION OF FLAVONOID 3'-HYDROXYLASE AND RELATED NUCLEIC ACID SEQUENCES

EXAMPLE 1-Plant Material

Petunia

The *Petunia hybrida* varieties used are presented in Table 4.

TABLE 4

Plant variety	Properties	Source/Reference
Old Glory Blue (OGB)	F_1 Hybrid	Ball Seed, USA
Old Glory Red (OGR)	F_1 Hybrid	Ball Seed, USA
V23	*An1, An2, An3, An4, An6, An8, An9, An10, ph1, Hf1, Hf2, ht1, Rt, po, Bl, Fl*	Wallroth *et al.* (1986) Doodeman *et al.* (1984)
R51	*An1, An2, An3, an4, An6, An8, An9, An10, An11, Ph1, hf1, hf2, Ht1, rt, Po, bl, fl*	Wallroth *et al.* (1986) Doodeman *et al.* (1984)
VR	V23 x R51 F_1 Hybrid	
SW63	*An1, An2, An3, an4, An6, An8, An9, An10, An11, Ph1, Ph2, Ph5, hf1, hf2, ht1, ht2, po, mf1, fl*	I.N.R.A., Dijon, Cedex France
Skr4	*An1, An2, An3, An4, An6, An11, hf1, hf2, ht1, Ph1, Ph2, Ph5, rt, Po, Mf1, Mf2, fl*	I.N.R.A., Dijon, Cedex France
Skr4 x SW63	F_1 Hybrid	

Plants were grown in specialised growth rooms with a 14 hour day length at a light intensity of 10,000 lux and a temperature of 22°C to 26°C.

Carnation

Flowers of *Dianthus caryophyllus* cv. Kortina Chanel were obtained from Van Wyk and Son

Flower Supply, Victoria.

Dianthus caryophyllus flowers were harvested at developmental stages defined as follows:

Stage 1: Closed bud, petals not visible.

Stage 2: Flower buds opening: tips of petals visible.

Stage 3: Tips of nearly all petals exposed. "Paint-brush stage".

Stage 4: Outer petals at 45° angle to stem.

Stage 5: Flower fully open.

Snapdragon

The *Antirrhinum majus* lines used were derived from the parental lines K16 (eos$^-$) and N8 (Eos$^+$). A strict correlation exists between F3'H activity and the Eos gene which is known to control the 3'-hydroxylation of flavones, flavonols and anthocyanins (Forkmann and Stotz, 1981). K16 is a homozygous recessive mutant lacking F3'H activity while N8 is wild type for F3'H activity. These lines are similar, though not isogenic. Both parental lines and the seed from a selfed (K16 x N8) F_1 plant were obtained from Dr C. Martin (John Innes Centre, Norwich, UK).

Arabidopsis

The *Arabidopsis thaliana* lines Columbia (Tt7), Landsberg erecta (Tt7) and NW88 (tt7) were obtained from the Nottingham Arabidopsis Stock Centre. Wild-type *A. thaliana* (Tt7) seeds have a characteristic brown colour. Seeds of tt7 mutants have pale brown seeds and the plants are characterized by a reduced anthocyanin content in leaves (Koornneef et al., 1982). Tt7 plants produce cyanidin while tt7 mutants accumulate pelargonidin, indicating that the Tt7 gene controls flavonoid 3'-hydroxylation.

Rose

Flowers of *Rosa hybrida* cv. Kardinal were obtained from Van Wyk and Son Flower Supply, Victoria.

Stages of *Rosa hybrida* flower development were defined as follows:

Stage 1: Unpigmented, tightly closed bud (10-12 mm high; 5 mm wide).

Stage 2: Pigmented, tightly closed bud (15 mm high ; 9 mm wide).

Stage 3: Pigmented, closed bud; sepals just beginning to open (20-25 mm high; 13-15 mm wide)

Stage 4: Flower bud beginning to open; petals heavily pigmented; sepals have separated (bud is 25-30 mm high and 18 mm wide).

Stage 5: Sepals completely unfolded; some curling. Petals are heavily pigmented and unfolding (bud is 30-33 mm high and 20 mm wide).

Chrysanthemum

Stages of *Chrysanthemum* flower development were defined as follows:

Stage 0: No visible flower bud.

Stage 1: Flower bud visible: florets completely covered by the bracts.

Stage 2: Flower buds opening: tips of florets visible.

Stage 3: Florets tightly overlapped.

Stage 4: Tips of nearly all florets exposed; outer florets opening but none horizontal.

Stage 5: Outer florets horizontal.

Stage 6: Flower approaching maturity.

EXAMPLE 2-Bacterial Strains

The *Escherichia coli* strains used were:

DH5α supE44, Δ(lacZYA-ArgF)U169, ø80lacZΔM15, hsdR17 (r_k-, m_k+), recA1, endA1, gyrA96, thi-1, relA1, deoR (Hanahan, 1983 and BRL, 1986).

XL1-Blue MRF' Δ(mcr A)183 , Δ(mcrCB-hsdSMR-mrr)173, endA1, supE44, thi-1,

recA1, gyrA96, relA1, lac[F' proAB, lacIqZ△M15, Tn10(Tet^r)]^c (Stratagene)

XL1-Blue supE44, hsdR17 (r_k-, m_k+), recA1, endA1, gyrA96, thi-1, relA1, lac[F' proAB, lacI^q, lacZ△M15, Tn10(tet^r)]

SOLR e14$^-$ (mcrA), △(mcrCB-hsdSMR-mrr)171, sbcC, recB, recJ, umuC::Tn5(kan^r), uvrC,lac, gyrA96, thi-1, relA1, [F'proAB, lacI^{q}Z△M15], Su$^-$ (non-suppressing) (Stratagene)

DH10 B(Zip) F$^-$mcrA, △(mrr-hsdRMS-mcrBC), ø80d lacZ△M15, △lacX74, deoR, recA1, araD139, △(ara, leu)7697, galU, galK1$^\lambda$, rspL, nupG

Y1090r- △lacU169, (△lon)?, araD139, strA, supF, mcrA, trpC22::Tn10(Tet^r) [pMC9 Amp^r, Tet^r], mcrB, hsdR

The disarmed *Agrobacterium tumefaciens* strain AGL0 (Lazo *et al.*, 1991) was obtained from R. Ludwig (Department of Biology, University of California, Santa Cruz, USA).

The cloning vector pBluescript was obtained from Stratagene.

Transformation of the *E. coli* strain DH5α cells was performed according to the method of Inoue *et al.* (1990).

EXAMPLE 3-General methods

^{32}P-Labelling of DNA Probes

DNA fragments (50 to 100 ng) were radioactively labelled with 50 μCi of [α-^{32}P]-dCTP using an oligolabelling kit (Bresatec). Unincorporated [α-^{32}P]-dCTP was removed by chromatography on a Sephadex G-50 (Fine) column.

DNA Sequence Analysis

DNA sequencing was performed using the PRISM™Ready Reaction Dye Primer Cycle Sequencing Kits from Applied Biosystems. The protocols supplied by the manufacturer were followed. The cycle sequencing reactions were performed using a Perkin Elmer PCR machine (GeneAmp PCR System9600) and run on an automated 373A DNA sequencer (Applied Biosystems).

Homology searches against Genbank, SWISS-PROT and EMBL databases were performed using the FASTA and TFASTA programs (Pearson and Lipman, 1988) or BLAST programs (Altschul *et al.*, 1990). Percentage sequence similarities were obtained using the LFASTA program (Pearson and Lipman, 1988). In all cases ktup values of 6 for nucleotide sequence comparisons and 2 for amino acid sequence comparisons were used, unless otherwise specified.

Multiple sequence alignments (ktup value of 2) were performed using the ClustalW program incorporated into the MacVector™6.0 application (Oxford Molecular Ltd.).

EXAMPLE 4- Isolation of a flavonoid 3'-hydroxylase (F3'H) cDNA clone corresponding to the Ht1 locus from *P. hybrida* cv. Old Glory Red

In order to isolate a cDNA clone that was linked to the Ht1 locus and that represented the flavonoid 3'-hydroxylase (F3'H) in the petunia flavonoid pathway, a petal cDNA library was prepared from RNA isolated from stages 1 to 3 of Old Glory Red (OGR) petunia flowers. OGR flowers contain cyanidin based pigments and have high levels of flavonoid 3'-hydroxylase activity. The OGR cDNA library was screened with a mixture of ^{32}P-labelled fragments isolated from three cytochrome P450 cDNA clones known to be involved in the flavonoid pathway and from one cytochrome P450 cDNA clone (651) that had flavonoid 3'-hydroxylase activity in yeast. These included a petunia cDNA clone representing the cinnamate-4-hydroxylase (C4H) and two petunia cDNA clones (coded by the Hf1 and Hf2 loci) representing flavonoid 3' 5'-hydroxylase (F3' 5'H) (Holton *et al.*, 1993).

Construction of Petunia cv. OGR cDNA library

Total RNA was isolated from the petal tissue of *P. hybrida* cv OGR stage 1 to 3 flowers using the method of Turpen and Griffith (1986). Poly(A)$^+$ RNA was selected from the total RNA, using oligotex-dT™ (Qiagen).

A ZAP-cDNA Gigapack III Gold Cloning kit (Stratagene) was used to construct a directional petal cDNA library in λZAP using 5 μg of poly(A)+ RNA isolated from stages 1 to 3 of OGR as template. The total number of recombinants obtained was 2.46×10^6.

After transfecting XL1-Blue MRF' cells, the packaged cDNA mixture was plated at 50,000 pfu per 15 cm diameter plate. The plates were incubated at 37°C for 8 hours, and the phage were eluted in 100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl pH 8.0, 0.01% (w/v) gelatin (Phage Storage Buffer (PSB)) (Sambrook *et al.*, 1989). Chloroform was added and the phage stored at 4°C as an amplified library.

100,000 pfu of the amplified library were plated onto NZY plates (Sambrook *et al.*, 1989) at a density of 10,000 pfu per 15 cm plate after transfecting XL1-Blue MRF' cells, and incubated at 37°C for 8 hours. After incubation at 4°C overnight, duplicate lifts were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Isolation of probes

F3'5'H probes

The two flavonoid 3', 5' hydroxylases corresponding to the Hf1 or Hf2 loci isolated as described in Holton *et al.* (1993) and US Patent Number 5,349,125, were used in the screening process.

C4H cDNA clones from petunia

A number of cytochrome P450 cDNA clones were isolated in the screening process used to

isolate the two flavonoid 3',5' hydroxylase cDNA clones corresponding to the Hf1 or Hf2 loci (Holton *et al.*, 1993; US Patent Number 5,349,125). One of these cDNA clones (F1) (contained in pCGP161) (Figure 2) was identified as representing a cinnamate 4-hydroxylase (C4H), based on sequence identity with a previously-characterised C4H clone from mung bean (Mizutani *et al.*, 1993). Sequence data was generated from 295 nucleotides at the 5' end of the petunia F1 cDNA clone. There was 83.1% similarity with the mung bean C4H clone over the 295 nucleotides sequenced and 93.9% similarity over the predicted amino acid sequence.

651 cDNA clone

The isolation and identification of the 651 cDNA clone contained in pCGP619 (Figure 5) was described in the International Patent Application, having publication number W093/20206. A protein extract of yeast containing the 651 cDNA clone under the control of the yeast glyceraldehyde-3-phosphate dehydrogenase promoter of pYE22m (Tanaka *et al.*, 1988) exhibited F3'H activity.

Screening of OGR Library

Prior to hybridization, the duplicate plaque lifts were washed in prewashing solution (50 mM Tris-HCl pH7.5, 1 M NaCl, 1 mM EDTA, 0.1% (w/v) sarcosine) at 65°C for 30 minutes; stripped in 0.4 M sodium hydroxide at 65°C for 30 minutes; then washed in a solution of 0.2 M Tris-HCl pH 8.0, 0.1 x SSC, 0.1% (w/v) SDS at 65°C for 30 minutes and finally rinsed in 2 x SSC, 1.0% (w/v) SDS.

The lifts from the OGR cDNA library were screened with ^{32}P-labelled fragments of (1) a 0.7 kb EcoRI/XhoI fragment from pCGP161 containing the C4H cDNA clone (Figure 2), (2) a 1.6 kb BspHI/FspI fragment from pCGP602 containing the Hf1 cDNA clone (Figure 3), (3) a 1.3 kb EcoRI/XhoI fragment and a 0.5 kb XhoI fragment from pCGP175 containing the coding region of the Hf2 cDNA clone (Figure 4) and (4) a 1.8 kb EcoRI/XhoI fragment pCGP619 containing the 651 cDNA clone (Figure 5).

Hybridization conditions included a prehybridization step in 10% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42°C for at least 1 hour. The ^{32}P-labelled fragments (each at 1x10^{6}cpm/mL) were then added to the hybridization solution and hybridization was continued at 42°C for a further 16 hours. The filters were then washed in 2 x SSC, 1% (w/v) SDS at 42°C for 2 x 1 hour and exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours.

Two hundred and thirty strongly hybridizing plaques were picked into PSB. Of these, 39 were rescreened to isolate purified plaques, using the hybridization conditions as described for the initial screening of the cDNA library. The plasmids contained in the λZAP bacteriophage vector were rescued and sequence data was generated from the 3' and 5' ends of the cDNA inserts. Based on sequence homology, 27 of the 39 were identical to the petunia cinnamate 4-hydroxylase cDNA clone, 2 of the 39 were identical to the Hf1 cDNA clone and 7 of the 39 did not represent cytochrome P450s. The remaining 3 cDNA clones (designated as OGR-27, OGR-38, OGR-39) represented "new" cytochrome P450s, compared to the cytochrome P450 clones used in the screening procedure, and were further characterised.

EXAMPLE 5 -Restriction Fragment Length Polymorphism (RFLP) analysis

There are two genetic loci in *P. hybrida*, Ht1 and Ht2, that control flavonoid 3'-hydroxylase activity (Tabak *et al.*, 1978; Wiering and de Vlaming, 1984). Ht1 is expressed in both the limb and the tube of *P. hybrida* flowers and gives rise to higher levels of F3'H activity than does Ht2 which is only expressed in the tube. The F3'H is able to convert dihydrokaempferol and naringenin to dihydroquercetin and eriodictyol, respectively. In a flower producing delphinidin-based pigments, F3'H activity is masked by the F3'5'H activity. Therefore, the F3'H/F3'5'H assay (Stotz and Forkmann, 1982) is useless in determining the presence or absence of F3'H activity. The enzyme flavonol synthase is able to convert dihydrokaempferol to kaempferol and dihydroquercetin to quercetin (Figure 1a). Myricetin, the 3', 5' hydroxylated flavonol, is produced at low levels in petunia flowers.

Therefore, analysing the flowers for the 3'-hydroxylated flavonol, quercetin, infers the presence of F3'H activity.

Restriction Fragment Length Polymorphism (RFLP) analysis of DNA isolated from individual plants in a VR (Ht1/ht1) x V23 (ht1/ht1) backcross was used to determine which, if any, of the cDNA clones representing P450s were linked to the Ht1 locus. Northern analysis of RNA isolated from these plants was also used to detect the presence or absence of a transcript in these lines.

Flowers from a VR (Ht1/ht1) x V23 (ht1/ht1) backcross population were analysed for the presence of the flavonols, kaempferol and quercetin. VR (Ht1/ht1) flowers accumulate quercetin and low levels of kaempferol while V23 (ht1/ht1) flowers accumulate kaempferol but little or no quercetin. Individual plants from the VR (Ht1/ht1) x V23 (ht1/ht1) backcross were designated as VR-like (Ht1/ht1), if a substantial level of quercetin was detected in the flower extracts, and V23-like (ht1/ht1), if little or no quercetin but substantial levels of kaempferol were detected in the flower extracts (see Figure 6).

Isolation of Genomic DNA

DNA was isolated from leaf tissue essentially as described by Dellaporta *et al.*, (1983). The DNA preparations were further purified by CsCl buoyant density centrifugation (Sambrook *et al.*, 1989).

Southern blots

The genomic DNA (10 μg) was digested for 16 hours with 60 units of EcoRI and electrophoresed through a 0.7% (w/v) agarose gel in a running buffer of TAE (40 mM Tris-acetate, 50 mM EDTA). The DNA was then denatured in denaturing solution (1.5 M NaCl/0.5 M NaOH) for 1 to 1.5 hours, neutralized in 0.5 M Tris-HCl (pH 7.5)/ 1.5 M NaCl for 2 to 3 hours and then transferred to a Hybond N (Amersham) filter in 20 x SSC.

RNA blots

Total RNA was isolated from the petal tissue of *P. hybrida* cv OGR stage 1 to 3 flowers using the method of Turpen and Griffith (1986).

RNA samples were electrophoresed through 2.2 M formaldehyde/1.2% (w/v) agarose gels using running buffer containing 40 mM morpholinopropanesulphonic acid (pH 7.0), 5 mM sodium acetate, 0.1 mM EDTA (pH 8.0). The RNA was transferred to Hybond-N filters (Amersham) as described by the manufacturer.

Hybridization and washing conditions

Southern and RNA blots were probed with ^{32}P-labelled cDNA fragment (10^8 cpm/μg, 2 x 10^6 cpm/mL). Prehybridizations (1 hour at 42°C) and hybridizations (16 hours at 42°C) were carried out in 50% (v/v) formamide, 1 M NaCl, 1% (w/v) SDS, 10% (w/v) dextran sulphate. Filters were washed in 2 x SSC, 1% (w/v) SDS at 65°C for 1 to 2 hours and then 0.2 x SSC, 1% (w/v) SDS at 65°C for 0.5 to 1 hour. Filters were exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours.

RFLP and Northern analysis of the cytochrome P450 fragments

RFLP analysis was used to investigate linkage of the genes corresponding to the OGR-27, OGR-38 and OGR-39 cDNA clones to the Ht1 locus.

^{32}P-labelled fragments of OGR-27, OGR-38 and OGR-39 cDNA clones were used to probe RNA blots and Southern blots of genomic DNA isolated from individual plants in the VR x V23 backcross population. Analysis of EcoRI digested genomic DNA isolated from a VR x V23 backcross population revealed a RFLP for the OGR-38 probe which was linked to Ht1. Furthermore, a much reduced level of transcript was detected in the V23-like lines, when compared with the high levels of transcript detected in VR-like lines (Figure 6).

The data provided strong evidence that the OGR-38 cDNA clone, contained in plasmid pCGP1805, corresponded to the Ht1 locus and represented a F3'H.

RFLP analysis of a V23 x R51 F_2 backcross

RFLP analysis was used to investigate linkage of the gene corresponding to the OGR-38 cDNA to known genetic loci.

The RFLP linkage analysis was performed using a Macintosh version 2.0 of the MapMaker mapping program (Du Pont) (Lander *et al*, 1987). A LOD score of 3.0 was used for the linkage threshold.

Analysis of EcoRI or XbaI digested genomic DNA isolated from a V23 x R51 F_2 population revealed a RFLP for the OGR-38 probe which was linked to PAc4. PAc4, a petunia actin cDNA clone (Baird and Meagher, 1987), is a molecular marker for chromosome III and is linked to the Htl locus (McLean *et al.*, 1990). There was co-segregation of the OGR-38 and PAc4 RFLPs for 36 out of 44 V23 x R51 F_2 plants. This represents a recombination frequency of 8% which is similar to a reported recombination frequency of 16% between the Htl locus and PAc4 (Cornu *et al.*, 1990).

Further characterisation of OGR-38

The developmental expression profiles in OGR petals, as well as in other OGR tissues, were determined by using the ^{32}P-labelled fragments of the OGR-38 cDNA insert as a probe against an RNA blot containing 20μg of total RNA isolated from each of the five petunia OGR petal developmental stages as well as from leaves, sepals, roots, stems, peduncles, ovaries, anthers and styles. The OGR-38 probe hybridized with a 1.8kb transcript that peaked at the younger stages of 1 to 3 of flower development. The OGR-38 hybridizing transcript was most abundant in the petals and ovaries and was also detected in the sepals, peduncles and anthers of the OGR plant. A low level of transcript was also detected in the stems. Under the conditions used, no hybridizing transcript was detected by Northern analysis of total RNA isolated from leaf, style or roots.

EXAMPLE 6- Complete sequence of OGR-38

The complete sequence of the OGR-38 cDNA clone (SEQ ID NO:1) was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook *et al.*, 1989). The sequence contained an open reading frame of 1536 bases which encodes a putative polypeptide of 512 amino acids.

The nucleotide and predicted amino acid sequences of OGR-38 (SEQ ID NO:1 and SEQ ID NO:2) were compared with those of the cytochrome P450 probes used in the screening process and with other petunia cytochrome P450 sequences (US Patent Number 5,349,125) using an lfasta alignment (Pearson and Lipman, 1988). The nucleotide sequence of OGR-38 was most similar to the nucleic acid sequence of the flavonoid 3' 5'-hydroxylases representing Hf1 and Hf2 loci from *P. hybrida* (Holton *et al.*, 1993). The Hf1 clone showed 59.6% similarity to the OGR-38 cDNA clone, over 1471 nucleotides, and 49.9% similarity, over 513 amino acids, while the Hf2 clone showed 59.1% similarity to the OGR-38 cDNA clone, over 1481 nucleotides, and 49.0% similarity, over 511 amino acids.

EXAMPLE 7- The F3'H assay of the Ht1 cDNA clone (OGR-38) expressed in yeast

Construction of pCGP1646

The plasmid pCGP1646 (Figure 7) was constructed by cloning the OGR-38 cDNA insert from pCGP1805 in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter of pYE22m (Tanaka *et al.*, 1988).

The plasmid pCGP1805 was linearised by digestion with Asp718. The overhanging 5' ends were "filled in" using DNA polymerase (Klenow fragment) according to standard protocols (Sambrook *et al.*, 1989). The 1.8 kb OGR-38 cDNA fragment was released upon digestion with SmaI. The cDNA fragment was isolated and purified using the Bresaclean kit (Bresatec) and ligated with blunted EcoRI ends of pYE22m. The plasmid pYE22m had been digested with EcoRI and the overhanging 5' ends were removed using DNA polymerase

(Klenow fragment) according to standard protocols (Sambrook *et al.*, 1989). The ligation was carried with the Amersham Ligation kit using 100ng of the 1.8 kb OGR-38 fragment and 150ng of the prepared yeast vector, pYE22m. Correct insertion of the insert in pYE22m was established by XhoI/SalI restriction enzyme analysis of the plasmid DNA isolated from ampicillin-resistant transformants.

Yeast transformation

The yeast strain G-1315 (Mat α, trp1) (Ashikari *et al.*, 1989) was transformed with pCGP1646 according to Ito *et al.* (1983). The transformants were selected by their ability to restore G-1315 to tryptophan prototrophy.

Preparation of yeast extracts for assay of F3'H activity

A single isolate of G-1315/pCGP1646 was used to inoculate 50 mL of Modified Burkholder's medium (20.0g/L dextrose, 2.0g/L L-asparagine, 1.5g/L KH_2PO_4, 0.5g/L $MgSO_4.7H_2O$, 0.33g/L $CaCl_2$, 2g/L $(NH_4)_2SO_4$, 0.1 mg/L KI, 0.92g/L $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.1g/L nitrilotriacetic acid, 0.99 mg/L $FeSO_4.7H_2O$, 1.25 mg/L EDTA, 5.47 mg/L $ZnSO_4.7H_2O$, 2.5 mg/L $FeSO_4.7H_2O$, 0.77 mg/L $MnSO_4.7H_2O$, 0.196 mg/L $CuSO_4.5H_2O$, 0.124 mg/L $Co(NH_4)_2(SO_4)_2.6H_2O$, 0.088 mg/L $Na_2B_4O_7.10H_2O$, 0.2 mg/L thiamine, 0.2 mg/L pyridoxine, 0.2 mg/L nicotinic acid, 0.2 mg/L pantothenate, 0.002 mg/L biotin, 10 mg/L inositol) which was subsequently incubated until the value at OD_{600} was 1.8 at 30°C. Cells were collected by centrifugation and resuspended in Buffer 1 [10 mM Tris-HCl buffer (pH 7.5) containing 2 M sorbitol, 0.1 mM DTT, 0.1 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mg yeast lytic enzyme/mL]. Following incubation for 1 hour at 30°C with gentle shaking, the cells were pelleted by centrifugation and washed in ice cold Buffer 2 [10 mM Tris-HCl (pH7.5) containing 0.65 M sorbitol, 0.1 mM DTT, 0.1 mM EDTA, 0.4 mM PMSF]. The cells were then resuspended in Buffer 2 and sonicated using six 15-second bursts with a Branson Sonifier 250 at duty cycle 30% and output control 10%. The sonicated suspension was centrifuged at 10,000 rpm for 30 minutes and the supernatant was centrifuged at 13,000 rpm for 90 minutes. The microsomal pellet was resuspended in assay buffer (100 mM potassium

phosphate (pH 8), 1 mM EDTA, 20 mM 2-mercaptoethanol) and 100 μL was assayed for activity.

F3'H Assay

F3'H enzyme activity was measured using a modified version of the method described by Stotz and Forkmann (1982). The assay reaction mixture typically contained 100 μL of yeast extract, 5 μL of 50 mM NADPH in assay buffer (100 mM potassium phosphate (pH8.0), 1 mM EDTA and 20 mM 2-mercaptoethanol) and 10 μCi of [^{3}H]- naringenin and was made up to a final volume of 210 μL with the assay buffer. Following incubation at 23°C for 2-16 hours, the reaction mixture was extracted with 0.5 mL of ethylacetate. The ethylacetate phase was dried under vacuum and then resuspended in 10 μL of ethylacetate. The tritiated flavonoid molecules were separated on cellulose thin layer plates (Merck Art 5577, Germany) using a chloroform: acetic acid: water (10:9:1 v/v) solvent system. The reaction products were localised by autoradiography and identified by comparison to non-radioactive naringenin and eriodictyol standards which were run alongside the reaction products and visualised under UV light.

F3'H activity was detected in extracts of G1315/pCGP1646, but not in extracts of non-transgenic yeast. From this it was concluded that the cDNA insert from pCGP1805 (OGR-38), which was linked to the Ht1 locus, encoded a F3'H.

EXAMPLE 8- Transient expression of the Ht1 cDNA clone (OGR-38) in plants

Construction of pCGP1867

Plasmid pCGP1867 (Figure 8) was constructed by cloning the cDNA insert from pCGP1805 in a "sense" orientation behind the Mac promoter (Comai *et al.*, 1990) of pCGP293 (Brugliera *et al.*, 1994). The plasmid pCGP1805 was digested with XbaI and KpnI to release the cDNA insert. The cDNA fragment was isolated and purified using the Bresaclean kit (Bresatec) and ligated with XbaI/KpnI ends of the pCGP293 binary vector. The ligation was carried out using the Amersham ligation kit. Correct insertion of the fragment in

pCGP1867 was established by XbaI/KpnI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

Transient expression of the Ht1 cDNA clone (OGR-38) in petunia petals

In order to rapidly determine whether the OGR-38 cDNA fragment in pCGP1867 represented a functional F3'H in plants, a transient expression study was established. Petals of the mutant *P. hybrida* line Skr4 x SW63 were bombarded with gold particles (1μm diameter) coated with pCGP1867 DNA.

Gold microcarriers were prewashed 3 times in 100% ethanol and resuspended in sterile water. For each shot, 1 μg of pCGP1867 DNA, 0.5 mg of gold microcarriers, 10 μL of 2.5 M $CaCl_2$ and 2 μL of 100 mM spermidine (free base) were mixed by vortexing for 2 minutes. The DNA coated gold particles were pelleted by centrifugation, washed twice with 100% ethanol and finally resuspended in 10 μL of 100% ethanol. The suspension was placed directly on the centre of the macrocarrier and allowed to dry.

Stages 1 and 2 of Skr4 x SW63 flowers were cut vertically into halves and partially embedded in MS solid media (3% (w/v) sucrose, 100 mg/L myo-inositol, 1xMS salts, 0.5 mg/L pyridoxine-HCl, 0.1 mg/L thiamine-HCl, 0.5 mg/L nicotinic acid and 2 mg/L glycine). The petals were placed so that the inside of the flower buds were facing upwards. A Biolistic PDS-1000/He System (Bio-Rad), using a Helium gas pressure of 900psi and a chamber vacuum of 28 inches of mercury, was used to project the gold microcarriers into the petal tissue. After 6-12 hours under lights in a controlled plant growth room at 22°C, red anthocyanin spots were observed on the upper epidermal layer of the petal tissue bombarded with pCGP1867-coated particles. No coloured spots were observed in control petal bombarded with gold particles alone. These results indicated that the OGR-38 cDNA clone under the control of the Mac promoter was functional, at least transiently, in petal tissue.

EXAMPLE 9- Stable expression of the Ht1 cDNA clone (OGR-38) in petunia petals- Complementation of a ht1/ht1 petunia cultivar

***A. tumefaciens* transformations**

The plasmid pCGP1867 (Figure 8) was introduced into the *Agrobacterium tumefaciens* strain AGL0 by adding 5 μg of plasmid DNA to 100 μL of competent AGL0 cells prepared by inoculating a 50 mL MG/L (Garfinkel and Nester, 1980) culture and growing for 16 hours with shaking at 28°C. The cells were then pelleted and resuspended in 0.5 mL of 85% (v/v) 100 mM $CaCl_2$/15% (v/v) glycerol. The DNA-*Agrobacterium* mixture was frozen by incubation in liquid N_2 for 2 minutes and then allowed to thaw by incubation at 37°C for 5 minutes. The DNA/bacterial mix was then placed on ice for a further 10 minutes. The cells were then mixed with 1 mL of LB (Sambrook *et al.*, 1989) media and incubated with shaking for 16 hours at 28°C. Cells of *A. tumefaciens* carrying pCGP1867 were selected on LB agar plates containing 10 μg/mL gentamycin. The presence of pCGP1867 was confirmed by Southern analysis of DNA isolated from the gentamycin-resistant transformants.

Petunia transformations

(a) Plant Material

Leaf tissue from mature plants of *P. hybrida* cv Skr4 x SW63 was treated in 1.25% (w/v) sodium hypochlorite for 2 minutes and then rinsed three times in sterile water. The leaf tissue was then cut into 25 mm^2 squares and precultured on MS media (Murashige and Skoog, 1962) supplemented with 0.05 mg/L kinetin and 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) for 24 hours.

(b) Co-cultivation of *Agrobacterium* and Petunia Tissue

A. tumefaciens strain AGL0 containing the binary vector pCGP1867 (Figure 11) was maintained at 4°C on MG/L agar plates with 100 mg/L gentamycin. A single colony was grown overnight in liquid medium containing 1% (w/v) Bacto-peptone, 0.5% (w/v) Bacto-

yeast extract and 1% (w/v) NaCl. A final concentration of 5 x 10^8 cells/mL was prepared the next day by dilution in liquid MS medium containing B5 vitamins (Gamborg *et al.*, 1968) and 3% (w/v) sucrose (BPM). The leaf discs were dipped for 2 minutes into BPM containing AGL0/pCGP1867. The leaf discs were then blotted dry and placed on co-cultivation media for 4 days. The co-cultivation medium consisted of SH medium (Schenk and Hildebrandt, 1972) supplemented with 0.05 mg/L kinetin and 1.0 mg/L 2,4-D and included a feeder layer of tobacco cell suspension spread over the co-cultivation medium with a filter paper placed on top of the tobacco cell suspension.

(c) Recovery of transgenic petunia plants

After co-cultivation, the leaf discs were transferred to selection medium (MS medium supplemented with 3% (w/v) sucrose, α-benzylaminopurine (BAP) 2 mg/L, 0.5 mg/L α-naphthalene acetic acid (NAA), kanamycin 300 mg/L, 350 mg/L cefotaxime and 0.3% (w/v) Gelrite Gellan Gum (Schweizerhall)). Regenerating explants were transferred to fresh selection medium after 4 weeks. Adventitious shoots which survived the kanamycin selection were isolated and transferred to BPM containing 100 mg/L kanamycin and 200 mg/L cefotaxime for root induction. All cultures were maintained under a 16 hour photoperiod (60 μmol. m-2, s-1 cool white fluorescent light) at 23± 2°C. When roots reached 2-3 cm in length the transgenic petunia plantlets were transferred to autoclaved Debco 51410/2 potting mix in 8 cm tubes. After 4 weeks, plants were replanted into 15 cm pots, using the same potting mix, and maintained at 23°C under a 14 hour photoperiod (300 μmol. m-2, s-1 mercury halide light).

EXAMPLE 10 -Transgenic plant phenotype analysis

pCGP1867 in Skr4 x SW63

Table 5 shows the various petal and pollen colour phenotypes obtained with Skr4 x SW63 plants transformed with the pCGP1867 plasmid. The transgenic plants #593A, 590A, 571A, 589A, 592A and 591A produced flowers with altered petal colour. Moreover, the anthers and pollen of the flowers from plants #593A, 590A, 589A, 592A and 591A were pink,

compared with those of the control Skr4 x SW63 plant, which were white. The change in anther and pollen colour, observed on introduction of plasmid pCGP1867 into Skr4 x SW63 petunia plants, was an unanticipated outcome. The colour codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

TABLE 5

Summary of petal, anther and pollen colours obtained in Skr4 xSW63 plants transformed with pCGP1867

Accession Number	Petal Limb Colour	RHSCC Code (petal limb)	Anther & Pollen Colour
Skr4 x SW63 control (594A)	very pale lilac	69B/73D	white
593A	dark pink	67B	pink
590A	dark pink and pink sectors	sectored 67B and 73A	pink
571A	pink	68A and B	pink
589A	dark pink	68A	pink
592A	pink and light pink sectors	68A and 68B	light pink
591A	dark pink	68A	pink
570A	very pale lilac	69B/73D	white

The expression of the introduced Ht1 cDNA in the Skr4 x SW63 hybrid had a marked effect on flower colour. The stamen tissue of the non-transgenic control is white, whereas the same tissue in most of the transgenic plants was pink. In addition, expression of the Ht1 cDNA in the Skr4 x SW63 hybrid conferred a dark pink hue to the corolla, which is normally very pale lilac.

EXAMPLE 11- Analysis of products

The anthocyanidins and flavonols produced in the petals and stamens (included the pollen, anthers and filaments) of the Skr4 x SW63 plants transformed with pCGP1867 were analysed by TLC.

Extraction of anthocyanins and flavonols

Prior to TLC analysis, the anthocyanin and flavonol molecules present in petal and stamen extracts were acid hydrolysed to remove glycosyl moieties from the anthocyanidin or flavonol core. Anthocyanidin and flavonol standards were used to help identify the compounds present in the floral extracts.

Anthocyanins and flavonols were extracted and hydrolysed by boiling between 100 to 200 mg of petal limbs, or five stamens, in 1 mL of 2 M hydrochloric acid for 30 minutes. The hydrolysed anthocyanins and flavonols were extracted with 200 μL of iso-amylalcohol. This mixture was then dried down under vacuum and resuspended in a smaller volume of methanol/1% (v/v) HCl. The volume of methanol/1% (v/v) HCl used was based on the initial fresh weight of the petal so that the relative levels of flavonoids in the petals could be estimated. Extracts from the stamens were resuspended in 1 μL of methanol/1% (v/v) HCl. A 1 μL aliquot of the extracts from the pCGP1867 in Skr4 x SW63 petals and stamens was spotted onto a TLC plate.

TLC analysis of floral extracts

Acid-hydrolysed floral extracts were run in a Forestal solvent system (HOAc:water:HCl; 30: 10: 3) (Markham, 1982). Table 6 shows the results of the TLC analysis of the anthocyanidins and flavonols present in some of the flowers and stamens of the transgenic Skr4 x SW63 petunia plants transformed with pCGP1867. Indicative relative amounts of the flavonols and anthocyanidins (designated with a "+" to "+++") were estimated by comparing the intensities of the spots observed on the TLC plate.

TABLE 6

Relative levels of anthocyanidins and flavonols detected in the petal limbs and stamens of Skr4 x SW63 plants transformed with pCGP1867.

Acc#	Petal Colour	Anthocyanidins			Flavonols	
		Malvidin	Cyanidin	Peonidin	Kaempferol	Quercetin
Skr4 x SW63 control petal limb	pale lilac	+/-	-	-	+	-
593A petal limb	dark pink	-	+	+++	-	++
571A petal limb	pink	-	+	+	-	+
589A petal limb	dark pink	-	+	++	-	++
570A petal limb	pale lilac	+/-	-	-	+	-
Skr4 x SW63 control stamens	white	-	-	-	+++	+
593A stamens	pink	-	-	++	-	+++

Introduction of the Ht1 cDNA clone into Skr4 x SW63 led to production of the 3'-hydroxylated flavonoids, quercetin, peonidin and some cyanidin in the petals. Peonidin is the methylated derivative of cyanidin (Figures 1a and 1b). Only kaempferol and a small amount of malvidin were detected in the non-transgenic Skr4 x SW63 control (Table 6). Although Skr4 x SW63 is homozygous recessive for both the Hf1 and Hf2 genes, these mutations do not completely block production of F3'5'H (see US Patent Number 5,349,125) and low levels of malvidin are produced to give the petal limb a pale lilac colour.

The stamens with the pink pollen and anthers produced by the transgenic plant #593A contained peonidin and quercetin, while the non-transgenic Skr4 x SW63 control with white pollen and anthers contained kaempferol and a low level of quercetin (Table 6).

The accumulation of the 3'-hydroxylated anthocyanidin, peonidin, in the petals and stamens of the transgenic Skr4 x SW63/pCGP1867 plants correlated with the pink and dark pink colours observed in the petals, anthers and pollen of the same plants.

Co-suppression of F3'H activity

The plasmid pCGP1867 was also introduced into *P. hybrida* cv. Old Glory Red (Ht1) in order to reduce the level of F3'H activity.

Petunia transformations were carried out as described in Example 9, above.

Two out of 38 trangenic plants produced flowers with an altered phenotype. OGR normally produces deep red flowers (RHSCC#46B). The two transgenic plants with altered floral colour produced flowers with a light pink or light red hue (RHSCC#54B and #53C).

Northern analysis on RNA isolated from flowers produced by four transgenic plants (the two transgenics with an altered phenotype and two transgenics with the usual deep red flowers) was performed to examine the level of OGR-38 transcripts. Ten micrograms of total petal RNA was separated on a 1.2% (w/v) agarose/formaldehyde gel (Sambrook *et al.* 1989) and transferred to HybondN nylon membrane (Amersham), as described previously. Petal RNA from a non-transformed OGR flower was also included as a control. ^{32}P-labelled fragments of the OGR-38 cDNA inserts were used to probe the RNA blot.

The OGR-38 probe detected transcripts of approximately 2.4 kb and 1.8 kb in the flowers of the transgenic plants. However, the level of both transcripts detected in the light pink and light red flowers was considerably lower than that detected in the deep red transgenic flowers. The endogenous 1.8 kb transcript was also detected in RNA from the non-transformed OGR flowers. In order to confirm that the 2.4kb transcript was from the introduced OGR-38 transgene, ^{32}P-labelled fragments of the *mas* terminator region were used to probe the same RNA blot. The *mas* probe detected the 2.4 kb transcript, suggesting that at least this transcript was derived from the introduced OGR-38 transgene.

Analysis of anthocyanin levels

The levels of anthocyanins in the control flowers and in the light pink transgenic flower were measured by spectrophotometric analysis.

Extraction of anthocyanins and flavonols

Anthocyanins and flavonols were extracted from petal limbs by incubating 200 to 300mg of petal limb in 2mL of methanol/1% (v/v) HCl for 16 hours at 4°C. Fifty μL of this solution was then added to 950μL of methanol/1% (v/v) HCl and the absorbance of the diluted solution at 530nm was determined. The anthocyanin level in nmoles per gram was determined using the formula: [(Abs (530 nm)/34,000) x volume of extraction buffer x dilution factor x 10^6] / weight in grams.

The light pink flower was found to contain approximately 915 nmoles of anthocyanin per gram of petal limb tissue whilst the control flower contained around 4000nmoles/gram.

These data suggest that introduction of the petunia F3'H (OGR-38) cDNA clone in a sense orientation into OGR plants leads to "co-suppression" (i.e. reduction) of both the endogenous and the transgenic F3'H transcripts. A correlation was observed between lighter flower colours, reduced anthocyanin production and reduced F3'H transcript level.

EXAMPLE 12- Isolation of a F3'H cDNA clone from *Dianthus caryophyllus*

In order to isolate a *Dianthus caryophylluss* (carnation) F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805 (described above), was used to screen a Carnation cv. Kortina Chanel petal cDNA library, under low stringency conditions.

Construction of Carnation cv. Kortina Chanel cDNA library

Twenty micrograms of total RNA isolated (as described previously) from stages 1, 2 and 3 of Kortina Chanel flowers was reverse transcribed in a 50 μL volume containing 1 x Superscript™ reaction buffer, 10 mM dithiothreitol (DTT), 500 μM dATP, 500 μM dGTP, 500 μM dTTP, 500 μM 5-methyl-dCTP, 2.8 μg Primer-Linker oligo from ZAP-cDNA Gigapack III Gold cloning kit (Stratagene) and 2 μL Superscript™ reverse transcriptase (BRL). The reaction mix was incubated at 37°C for 60 minutes, then placed on ice. A

ZAP-cDNA Gigapack III Gold Cloning kit (Stratagene) was used to complete the library construction. The total number of recombinants was 2.4 x 10^6.

A total of 200,000 pfu of the packaged cDNA was plated at 10,000 pfu per 15 cm diameter plate after transfecting XL1-Blue MRF' cells. The plates were incubated at 37°C for 8 hours, then stored overnight at 4°C. Duplicate lifts were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Screening of Kortina Chanel petal cDNA library for a F3'H cDNA clone

Prior to hybridization, the duplicate plaque lifts were treated as described previously. The duplicate lifts from the Kortina Chanel petal cDNA library were screened with ^{32}P-labelled fragments of the 1.8 kb EcoRI/XhoI insert from pCGP1805. Low stringency conditions, as described for the screening of the petunia OGR cDNA library, were used.

One strongly-hybridizing plaque was picked into PSB and rescreened as detailed above to isolate purified plaques. The plasmid contained in the lZAP bacteriophage vector was rescued and named pCGP1807.

The KC-1 cDNA insert contained in pCGP1807 was released upon digestion with EcoRI/XhoI and is around 2 kb. The complete sequence of the KC-1 cDNA clone was determined by compilation of sequence from subclones of the KC-1 cDNA insert. (Partial sequence covering 458 nucleotides had previously been generated from a 800 bp KpnI fragment covering the 3' region of KC-1 which was subcloned into pBluescript to give pCGP1808.) The complete sequence (SEQ ID NO:3) contained an open reading frame of 1508 bases which encodes a putative polypeptide of 500 amino acids (SEQ ID NO:4).

The nucleotide and predicted amino acid sequences of the carnation KC-1 cDNA clone were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The sequences of the carnation KC-1 cDNA clone (SEQ ID NO:3 and 4) showed 67.3% similarity, over 1555 nucleotides, and 71.5 % similarity, over 488 amino acids, to

that of the petunia OGR-38-F3'H-cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

EXAMPLE 13- Stable expression of the carnation F3'H cDNA (KC-1) clone in petunia petals- Complementation of a ht1/ht1 petunia cultivar

Preparation of pCGP1810

Plasmid pCGP1810 (Figure 9) was constructed by cloning the cDNA insert from pCGP1807 in a "sense" orientation behind the Mac promoter (Comai *et al.*, 1990) of pCGP90 (US Patent Number 5,349,125), a pCGP293 based construct (Brugliera *et al.*, 1994). The plasmid pCGP1807 was digested with BamHI and ApaI to release the KC-1 cDNA insert. The cDNA fragment was isolated and purified using the Bresaclean kit (Bresatec). The pCGP90 binary vector was digested with BamHI and ApaI to release the linearised vector and the Hf1 cDNA insert. The linearised vector was isolated and purified using the Bresaclean kit (Bresatec) and ligated with BamHI/ApaI ends of the KC-1 cDNA clone. The ligation was carried out using the Amersham ligation. Correct insertion of the insert in pCGP1810 was established by BamHI/ApaI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

The binary vector pCGP1810 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP1810/AGL0 cells were subsequently used to transform Skr4 x SW63 petunia plants (also described in Example 9), to test for stable expression and activity of the enzyme encoded by the gene corresponding to the KC-1 cDNA clone.

EXAMPLE 14-Transgenic plant phenotype analysis

pCGP1810 in Skr4 x SW63

The expression of the introduced KC-1 cDNA in the Skr4 x SW63 hybrid had a marked effect on flower colour. Ten of the twelve transgenic plants transformed with pCGP1810 produced flowers with an altered petal colour (RHSCC# 73A), compared with the Skr4 x Sw63 control (RHSCC# 74C). Moreover the anthers and pollen of the transgenic flowers were pink, compared with those of the control Skr4 x SW63 plant, which were white.. In addition, expression of the KC-1 cDNA in the Skr4 x SW63 hybrid conferred a dark pink hue to the corolla, which is normally pale lilac. The colour codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

Acid-hydrolysed floral extracts (see Example 11) were run in a Forestal solvent system (HOAc:water:HCl; 30: 10: 3) (Markham, 1982). The 3' hydroxylated flavonoids, peonidin and quercetin, were readily detected in the petal limbs of the transgenic plants. Only kaempferol and a small amount of malvidin were detected in the non-transgenic Skr4 x SW63 control.

The accumulation of the 3'-hydroxylated anthocyanidin, peonidin, in the petals of the transgenic Skr4 x SW63/pCGP1810 plants correlated with the dark pink colours observed in the petals of the same plants.

Construction of pCGP1813

Plasmid pCGP1811 was constructed by cloning the cDNA insert from pCGP1807 in a "sense" orientation behind the Mac promoter (Comai *et al.*, 1990) of pCGP1958. The plasmid pCGP1958 contains the Mac promoter and mannopine synthase (*mas*)(Comai *et al.*, 1990) terminator in a pUC19 backbone. The plasmid pCGP1807 was digested with PstI and XhoI to release the cDNA insert. The overhanging 5' ends were filled in using DNA

polymerase (Klenow fragment) (Sambrook *et al.*, 1989). The cDNA fragment was isolated and purified using the Bresaclean kit (Bresatec) and ligated with SmaI ends of the pCGP1958 vector to produce pCGP1811.

The plasmid pCGP1811 was subsequently digested with PstI to release the expression cassette containing the Mac promoter driving the KC-1 cDNA with a *mas* terminator, all of which were contained on a 4kb fragment. The expression cassette was isolated and ligated with PstI ends of the pWTT2132 binary vector (DNA Plant Technology Corporation; Oakland, California) to produce pCGP1813 (Figure 10).

Transformation of *Dianthus caryophyllus* cv. Kortina Chanel with the Carnation F3'H cDNA clone.

The binary vector pCGP1813 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP1813/AGL0 cells were used to transform carnation plants, to reduce the amount of 3'-hydroxylated flavonoids.

(a) Plant Material

Dianthus caryophyllus (cv. Kortina Chanel) cuttings were obtained from Van Wyk and Son Flower Supply, Victoria, Australia. The outer leaves were removed and the cuttings were sterilised briefly in 70% v/v ethanol followed by 1.25% w/v sodium hypochlorite (with Tween 20) for 6 min and rinsed three times with sterile water. All the visible leaves and axillary buds were removed under the dissecting microscope before co-cultivation.

(b) Co-cultivation of *Agrobacterium* and *Dianthus* Tissue

Agrobacterium tumefaciens strain AGL0 (Lazo *et al.*, 1991), containing the binary vector pCGP1813, was maintained at 4°C on LB agar plates with 50 mg/L tetracycline. A single colony was grown overnight in liquid LB broth containing 50 mg/L tetracycline and diluted to 5 x 10^8 cells/mL next day before inoculation. *Dianthus* stem tissue was co-cultivated with *Agrobacterium* for 5 days on MS medium supplemented with 3% w/v sucrose, 0.5 mg/L BAP, 0.5 mg/L 2,4-dichlorophenoxy-acetic acid (2,4-D), 100 mM acetosyringone

and 0.25% w/v Gelrite (pH 5.7).

(c) **Recovery of Transgenic *Dianthus* Plants**

For selection of transformed stem tissue, the top 6-8 mm of each co-cultivated stem was cut into 3-4 mm segments, which were then transferred to MS medium (Murashige and Skoog, 1962) supplemented with 0.3% w/v sucrose, 0.5 mg/L BAP, 0.5 mg/L 2,4-D, 1 μg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite. After 2 weeks, explants were transferred to fresh MS medium containing 3% sucrose, 0.16 mg/L thidiazuron (TDZ), 0.5 mg/L indole-3-butyric acid (IBA), 2 μg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite and care was taken at this stage to remove axillary shoots from stem explants. After 3 weeks, healthy adventitious shoots were transferred to hormone free MS medium containing 3% w/v sucrose, 5 μg/L chlorsulfuron, 500 mg/L ticarcillin, 0.25% w/v Gelrite. Shoots which survived 5 μg/L chlorsulfuron were transferred to the same medium for shoot elongation.

Elongated shoots were transferred to hormone-free MS medium containing 5 μg/L chlorsulfuron, 500 mg/L ticarcillin and 0.4% w/v Gelrite, in glass jars, for normalisation and root production. All cultures were maintained under a 16 hour photoperiod (120 $mE/m^2/s$ cool white fluorescent light) at 23± 2°C. Normalised plantlets, approximately 1.5-2 cm tall, were transferred to soil (75% perlite/25% peat) for acclimation at 23°C under a 14 hour photoperiod (200 $mE/m^2/s$ mercury halide light) for 3-4 weeks. Plants were fertilised with a carnation mix containing 1g/L $CaNO_3$ and 0.75 g/L of a mixture of microelements plus N:P:K in the ratio 4.7:3.5: 29.2.

EXAMPLE 15 -Isolation of a F3'H cDNA clone from *Antirrhinum majus* (Snapdragon) using a differential display approach

A novel approach was employed to isolate a cDNA sequence encoding F3'H from *Antirrhinum majus* (snapdragon). Modified methods based on the protocols for (i) isolation of plant cytochrome P450 sequences using redundant oligonucleotides (Holton *et al.* 1993)

and (ii) differential display of eukaryotic messenger RNA (Liang and Pardee, 1992) were combined, to compare the petal cytochrome P450 transcript populations between wild type (Eos$^+$) and F3'H mutant (eos$^-$)snapdragon lines. Direct cloning of differentially expressed cDNA fragments allowed their further characterisation by Northern, RFLP and sequence analysis to identify putative F3'H encoding sequences. A full-length cDNA was obtained using the RACE protocol of Frohman *et al.* (1988) and the clone was shown to encode a functional F3'H following both transient and stable expression in petunia petal cells.

Plant Material

The *Antirrhinum majus* lines used were derived from the parental lines K16 (eos$^-$) and N8 (Eos$^+$). K16 is a homozygous recessive mutant lacking F3'H activity, while N8 is wild type for F3'H activity. These lines are similar, though not isogenic. The seed of capsule E228^2 from the selfed K16 x N8 F_1 plant (#E228) was germinated and the resultant plants (K16 x N8 F_2 plants) were scored for the presence or absence of cyanidin, a product of F3'H activity (see Figures 1a and 1b). The presence of cyanidin could be scored visually, as the flowers were a crimson colour, unlike the mutant plants which were a pink colour (from pelargonidin-derived pigments). The accuracy of the visual scoring was confirmed by TLC analysis of the petal anthocyanins, carried out as described in Example 11.

Of 13 plants raised from the E228^2 seed, 9 (#3, #4, #5, #6, #7, #9, #10, #12, #15) produced flowers with cyanidin (Eos$^+$/Eos$^+$ and Eos$^+$/eos$^-$) while 4 (#8, #11, #13, #14) produced only pelargonidin-derived pigments (eos$^-$/eos$^-$).

Synthesis of cDNA

Total RNA was isolated from the leaves of plant #13 and petal tissue of plants #3, #5, and #12 of the *A. majus* K16 x N8 F_2 segregating population (E228^2) using the method of Turpen and Griffith (1986). Contaminating DNA was removed by treating 50 μg total RNA with 1 unit RQ1 RNase-free DNase (Promega) in the presence of 40 units RNasin® ribonuclease inhibitor (Promega) for 3 hours at 37°C in a buffer recommended by the manufacturers. The RNA was then further purified by extraction with phenol/chloroform/iso-

amyl alcohol (25:24:1) and subsequent ethanol precipitation.

Anchored poly(T) oligonucleotides, complementary to the upstream region of the polyadenylation sequence, were used to prime cDNA synthesis from *A. majus* petal and leaf RNA. The oligonucleotide sequences synthesized were (5'-3'):

polyT-anchA	TTTTTTTTTTTTTTTTA	SEQ ID NO:27
polyT-anchC	TTTTTTTTTTTTTTTTC	SEQ ID NO:28
polyT-anchG	TTTTTTTTTTTTTTTTG	SEQ ID NO:29

Two micrograms of total RNA and 100 pmol of the appropriate priming oligonucleotide were heated to 70°C for 10 minutes, then chilled on ice. The RNA/primer hybrids were then added to a reaction containing 20 units RNasin® (Promega), 25 nM each dNTP, 10 mM DTT and 1x Superscript buffer (BRL). This reaction was heated at 37°C for 2 minutes, then 200 units of Superscript™ reverse transcriptase (BRL) were added and the reaction allowed to proceed for 75 minutes, after which the reverse transcriptase was inactivated by heating the mixture at 95°C for 20 minutes.

Amplification of cytochrome P450 sequences using PCR

Cytochrome P450 sequences were amplified using redundant oligonucleotides (designed to be complementary to conserved regions near the 3' end of plant cytochrome P450 coding sequences) and polyT anchored oligonucleotides. A similar approach was previously used to generate cytochrome P450 sequences from *Petunia hybrida* and is described in US Patent Number 5,349,125.

Four oligonucleotides (referred to as upstream primers) were synthesized. These were derived from conserved amino acid regions in plant cytochrome P450 sequences. The oligonucleotides (written 5' to 3') were as follows:

WAIGRDP TGG GCI ATI GGI (A/C)GI GA(T/C) CC

SEQ ID NO:30	SEQ ID NO:31
FRPERF	AGG AAT T(T/C)(A/C) GIC CIG A(A/G)(A/C) GIT T
SEQ ID NO:32	SEQ ID NO:33
Pet Haem-New	CCI TT(T/C) GGI GCI GGI (A/C)GI (A/C)GI ATI TG(T/G) (C/G)CI GG
	SEQ ID NO:34
EFXPERF	GAI TT(T/C) III CCI GAI (A/C)GI TT
SEQ ID NO:35	SEQ ID NO:36

The upstream primers were used with each of the polyT anchored oligonucleotides to generate cytochrome P450 sequences in polymerase chain reactions using cDNA as a template. Fifty pmol of each oligonucleotide was combined with 2 μM of each dNTP, 1.5 mM $MgCl_2$, 1x PCR buffer (Perkin Elmer), 5 μCi α-[^{33}P] dATP (Bresatec, 1500 Ci/mmol), 2.5 units AmpliTaq® DNA polymerase (Perkin Elmer) and 1/10th of the polyT-anchor primed cDNA reaction (from above). Reaction mixes (50 μL) were cycled 40 times between 94°C for 15 seconds, 42°C for 15 seconds, and 70°C for 45 seconds, following an initial 2 minute denaturation step at 94°C. Cycling reactions were performed using a Perkin Elmer 9600 Gene Amp Thermal Cycler.

DNA sequences were amplified using each upstream primer/anchored primer combination and the appropriately-primed cDNA template. Each primer combination was used with the cDNA from the petals of the E228^2 plants #3 and #5 (cyanidin-producing flowers) and #12 (non-cyanidin producing flowers). Reactions incorporating leaf cDNA from plant #13 (cyanidin-producing flowers) were also included, as negative controls, because F3'H activity is not present at a significant level in healthy, unstressed leaf tissues.

Differential display of cytochrome P450 sequences

^{33}P-labelled PCR fragments were visualised following separation on a 5% (w/v) polyacrylamide/urea denaturing gel (Sambrook *et al.* 1989). A ^{33}P-labelled M13mp18 sequencing ladder was included on the gel to serve as a size marker. The sequencing gel was dried onto Whatman 3MM paper and exposed to Kodak XAR film at room temperature.

Comparison of bands between cyanidin-producing petal samples and the non-cyanidin petal sample revealed 11 bands which represent mRNAs exclusively present in the cyanidin-producing petals. Of these 11 bands, only two were also present (at a reduced intensity) in the leaf sample.

Isolation and cloning of PCR fragments from sequencing gel

PCR products were purified from the dried sequencing gel and reamplified by the method described by Liang *et al.* (1993). Amplified cDNAs were purified, following electrophoretic separation on a 1.2% (w/v) agarose/TAE gel, using a Bresaclean kit (Bresatec). The purified fragments were then directly ligated into either commercially-prepared pCR-Script™vector (Stratagene) or EcoRV-linearised pBluescript® (Stratagene) which had been T-tailed using the protocol of Marchuk *et al.* (1990).

Sequence of F3'H PCR products

Each of the eleven cloned differential display PCR products (with inserts not exceeding 500 bp) was sequenced on both strands and compared to other known cytochrome P450 sequences involved in anthocyanin biosynthesis, using the FASTA algorithm of Pearson and Lipman (1988).

Of the eleven cDNAs cloned, two (Am1Gb and Am3Ga) displayed strong homology with the petunia OGR-38 F3'H sequence (Examples 4 to 11) and the F3'5'H sequences (Holton *et al.*, 1993). Conserved sequences between clones Am1Gb and Am3Ga suggested that they represented overlapping fragments of the same mRNA. Clone Am3Ga extends from the sequence encoding the haem-binding region of the molecule (as recognised by the "Pet Haem-New" oligonucleotide; SEQ ID NO:34) to the polyadenylation sequence. Clone

Am1Gb extends from the cytochrome P450 sequence encoding the conserved "WAIGRDP" amino acid motif (complementary to primer 1; SEQ ID NO:30 and SEQ ID NO:31) to an area in the 3' untranslated region which was spuriously recognised by the primer 1 ("WAIGRDP") oligonucleotide.

EXAMPLE 16- RFLP analysis of cytochrome P450 cDNAs

Restriction fragment length polymorphism (RFLP) analysis was again used to investigate linkage of the gene corresponding to cDNA clone Am3Ga to the presence, or absence, of cyanidin-producing activity in petals. A ^{32}P-labelled insert of Am3Ga was used to probe Southern blots of genomic DNA isolated from K16 x N8 F_2 segregating plants as well as the parental K16 and N8 lines. Analysis of EcoRV-digested genomic DNA from 13 plants of the K16 x N8 F_2 segregating population revealed hybridization only to the sequences of N8 and the K16 x N8 F_2 segregating lines which displayed floral cyanidin production (Figure 11). The K16 x N8 F_2 plants which produced only pelargonidin-derived pigments in their petals (including parental line, K16) showed no specific hybridization (Figure 11, lanes 2, 8, 11, 13, 14). These data indicate a possible deletion of the genomic sequences corresponding to Am3Ga in the mutant K16 plant and, therefore, at least a partial deletion of the F3'H gene in this line.

EXAMPLE 17- Northern analysis of cytochrome P450 cDNAs

Northern analysis was used to confirm the expression profiles of the putative cytochrome P450 fragments as shown by differential display. Ten micrograms of total petal RNA from eight of the K16 x N8 F_2 segregating population was separated on a 1.2% (w/v) agarose/formaldehyde gel (Sambrook *et al.* 1989) and transferred to HybondN nylon membrane (Amersham). Leaf RNA from the cyanidin-producing plant #13 was also included as a negative control in the Northern analysis. ^{32}P-labelled fragments of the cDNA insert from clone Am3Ga was used to probe the RNA blot.

The Am3Ga probe recognised an approximately 1.8 kb transcript which was only detectable in the petals of cyanidin-producing plants (plants #1, #3, #4, #5, #8). No transcript was detectable in the pelargonidin-producing petals (plants #6, #11, #12) or in the leaf sample from plant #13 (Figure 12).

These data, taken with those of the RFLP analysis, provide strong evidence that Am3Ga clone represents a cytochrome P450 gene which is responsible for F3'H activity in snapdragon. The total lack of a detectable transcript in the petals of non-cyanidin-producing lines supports the findings of the RFLP analysis, that the loss of cyanidin-producing activity in the K16 line (and the homozygous recessive plants of the K16 x N8 F_2 segregating population) is the result of a deletion in the F3'H structural gene.

EXAMPLE 18- Isolation of a full-length snapdragon F3'H cDNA

The Rapid Amplification of cDNA Ends (RACE) protocol of Frohman *et al.* (1988) was employed to isolate a full-length F3'H cDNA clone using sequence knowledge of the partial Am3Ga clone. A gene-specific primer ("SnapredRace A" -complementary to Am3Ga sequences 361 to 334) was synthesized to allow reverse transcription from petal RNA. A 3' amplification primer ("SnapredRace C" -complementary to Am3Ga (3'UTR) sequences 283 to 259) was also synthesized to bind just upstream of "SnapredRace A". A "poly(C)" primer was used to amplify sequences from the 5' end of the cDNA molecule.

The sequences of the oligonucleotides used were (written 5'-3'):

Snapred Race A CCA CAC GAG TAG TTT TGG CAT TTG ACC C
SEQ ID NO:37

Snapred Race C GTC TTG GAC ATC ACA CTT CAA TCT G
SEQ ID NO:38

PolyC race	CCG AAT TCC CCC CCC CC

SEQ ID NO:39

"Snapred Race A-primed" petal cDNA was poly(G)-tailed and a 5' cDNA fragment amplified with primers "Snapred Race C" and "PolyC race" using the method of Frohman *et al.* (1988). Pfu DNA polymerase (0.15 unit) (Stratagene) was combined with 2.5 units AmpliTaq® DNA polymerase (Perkin Elmer) to increase the fidelity of the PCR reaction.

The resultant 1.71 kb DNA fragment (sdF3'H) was cloned directly into EcoRV-linearised pBluescript® (Stratagene) vector which had been T-tailed using the protocol of Marchuk *et al.* (1990). This plasmid was named pCGP246.

EXAMPLE 19- Complete sequence of snapdragon F3'H

Convenient restriction sites within the sdF3'H cDNA sequence of pCGP246 were exploited to generate a series of short overlapping subclones in the plasmid vector pUC19. The sequence of each of these subclones was compiled to provide the sequence of the entire sdF3'H RACE cDNA. The sdF3'H cDNA sequence was coupled with that from clone Am3Ga to provide the entire sequence of a snapdragon F3'H cDNA (SEQ ID NO:5). It contains an open reading frame of 1711 bases which encodes a putative polypeptide of 512 amino acids (SEQ ID NO:6).

The nucleotide and predicted amino acid sequences of the snapdragon sdF3'H clone were compared with: those of the petunia OGR-38 cDNA clone (SEQ ID NO:1 and SEQ ID NO:2); the petunia F3'5'H cDNA clones Hf1 and Hf2; and other petunia cytochrome P450 sequences isolated previously (US Patent Number 5,349,125). The sequence of sdF3'H was most similar to that of the petunia F3'H cDNA clone (OGR-38) representing the Ht1 locus from *P. hybrida*, having 69% similarity at the nucleic acid level, over 1573 nucleotides, and 72.2% similarity at the amino acid level, over 507 amino acids.

The Hf1 clone showed 57.3% similarity, over 1563 nucleotides and 49.3% similarity, over 491 amino acids, to the snapdragon sdF3'H clone, while the Hf2 clone showed 57.7% similarity, over 1488 nucleotides, and 50.8% similarity, over 508 amino acids, to the snapdragon sdF3'H clone.

The snapdragon sdF3'H sequence contains two "in frame" ATG codons which could act to initiate translation. Initiation from the first of these codons (position 91 of SEQ ID NO:5) gives a protein with an additional 10 N-terminal amino acids and would be favoured according to the scanning model for translation (Kozak, 1989).

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

EXAMPLE 20- Transient expression of sdF3'H in plants

Construction of pCGP250

Plasmid pCGP250 (Figure 13) was created by cloning the entire sdF3'H RACE cDNA insert (from position 1 to 1711 (SEQ ID NO:5)) from pCGP246 in the "sense" orientation behind the Mac promoter (Comai *et al.*, 1990) of pCGP293 (Brugliera *et al.*, 1994). The plasmid pCGP246 was digested with EcoRI to release the cDNA insert. The cDNA fragment was blunt-ended by repairing the overhangs with the Klenow fragment of DNA polymerase I (Sambrook *et al.*, 1989) and purified, following agarose gel electrophoresis, using a Bresaclean kit (Bresatec). The blunt cDNA fragment was then ligated into the binary vector pCGP293, which had been linearised with XbaI and blunt-ended using the Klenow fragment of DNA polymerase I. The ligation was carried out using the Amersham ligation kit. Correct insertion of the insert in pCGP250 was established by BamHI and PstI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

Construction of pCGP231

Plasmid pCGP231 (Figure 14) was created by cloning the RACE cDNA insert from pCGP246, downstream of the first "in-frame" ATG codon (from position 104 to 1711 (SEQ ID NO:5), in the "sense" orientation behind the Mac promoter (Comai *et al.*, 1990) of pCGP293 (Brugliera *et al.*, 1994). The plasmid pCGP246 was digested with SspI (which recognises a site between the candidate ATG codons) and SmaI (with a site in the vector polylinker sequence) to release a blunt-ended cDNA fragment which includes the entire coding region downstream from the second putative initiation codon. The cDNA fragment was then ligated into the binary vector pCGP293, which had been linearised with XbaI and blunt-ended using the Klenow fragment of DNA polymerase I. The ligation was carried out using the Amersham ligation kit. Correct insertion of the insert in pCGP231 was established by BamHI and PstI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

Transient Expression Studies

To determine rapidly whether the pCGP246 sequences in pCGP231 and pCGP250 encoded active flavonoid 3'-hydroxylases in plants, a transient expression study was undertaken. Petals of the mutant *P. hybrida* line Skr4 X SW63 were bombarded with gold particles (1μm diameter) coated with either pCGP231 or pCGP250 plasmid DNA, using the method described in Example 8.

After 6-12 hours under lights in a controlled plant growth room at 22°C, red anthocyanin spots were observed on the surface of the petal tissue bombarded with pCGP231 coated particles. No coloured spots were observed in petals bombarded with pCGP250 or control petals bombarded with gold particles alone. These results indicated that the pCGP246 coding region (starting at the second ATG, position 121 of SEQ ID NO:5), under the control of the Mac promoter, was functional in petal tissue.

EXAMPLE 21- Stable expression of the snapdragon F3'H cDNA clone in petunia petals- Complementation of a ht1/ht1 petunia cultivar

The binary vectors pCGP250 and pCGP231 were introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP250/AGL0 and pCGP231/AGL0 cells were used to transform Skr4 x SW63 petunia plants (also described in Example 9), to test for stable expression and activity of the enzyme encoded by the gene corresponding to the snapdragon sdF3'H cDNA clone.

Three of the nine transgenic plants transformed with pCGP250 produced flowers with a slightly-altered petal colour (RHSCC# 73A), compared with the Skr4 x Sw63 control (RHSCC# 75C). Of the 11 transgenic plants transformed with pCGP231, one plant produced flowers with an altered petal colour (RHSCC# 73B). The anthers and pollen of the transgenic flowers were also white, as in the control. The codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

TLC analysis of floral extracts

Acid-hydrolysed floral extracts (see Example 11) were run in a Forestal solvent system (HOAc:water:HCl; 30: 10: 3) (Markham, 1982). Introduction of the sdF3'H cDNA clone into Skr4 x SW63 led to the production of increased levels of the 3'-hydroxylated flavonoid, peonidin, in the petals. Peonidin is the methylated derivative of cyanidin (Figures 1a and 1b).

EXAMPLE 22- Isolation of a F3'H cDNA clone from *Arabidopsis thaliana* using a PCR approach

In order to isolate a cDNA clone representing flavonoid 3'-hydroxylase from *Arabidopsis thaliana*, PCR fragments were generated using primers from the conserved regions of cytochrome P450s. One PCR product (p58092.13) was found to have high sequence similarity with the petunia OGR-38 and snapdragon F3'H cDNA clones. The PCR fragment

was then used, together with the Ht1 cDNA insert (OGR-38) from pCGP1805, to screen an *A. thaliana* cDNA library.

Design of oligonucleotides

Degenerate oligonucleotides for PCR DNA amplification were designed from the consensus amino acid sequence of *Petunia hybrida* cytochrome P450 partial sequences situated near the haem-binding domain. Primer degeneracy was established by the inclusion of deoxyinosine (designated as I below) in the third base of each codon (deoxyinosine base pairs with similar efficiency to A, T, G, and C), and the inclusion of alternate bases where the consensus sequences were non-specific. Thus, the amino-terminal directional primer "Pet Haem" (Petunia haem-binding domain), containing the cysteine residue codon crucial for haem binding, and the upstream primer "WAIGRDP" (See also Example 15) were designed.

WAIGRDP TGG GCI ATI GGI (A/C)GI GA(T/C) CC
SEQ ID NO:30 SEQ ID NO:31

Pet Haem CCI GG(A/G) CAI ATI C(G/T)(C/T) (C/T)TI CCI GCI CC(A/G) AAI GG
SEQ ID NO:40

Generation of cytochrome P450 sequences using PCR

Genomic DNA was isolated from *A. thaliana* ecotype Columbia, using the method described by Dellaporta *et al.* (1987). Polymerase chain reactions for amplification of cytochrome P450 homologues typically contained 100-200 ng of Columbia genomic DNA, 10 mM Tris-HCl (pH8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.2 mM each dNTP, 312 ng "WAIGRDP" and 484 ng "Pet Haem" and 1.25 units Taq polymerase (Cetus). Reaction mixes (50 μL) were cycled 40 times between 95°C for 50 seconds, 45°C for 50 seconds and 72°C for 45 seconds.

The expected size of specific PCR amplification products, using the "WAIGRDP" and "Pet Haem" primers on a typical P450 gene template, without an intron, is approximately 150

base pairs. PCR fragments of approximately 140 to 155 base pairs were isolated and purified using the Mermaid® kit (BIO 101). The PCR fragments were re-amplified to obtain enough product for cloning and then end-repaired using Pfu DNA polymerase and finally cloned into pCR-Script™Direct SK(+) (Stratagene). The ligated DNA was then used to transform competent DH5α cells (Inoue *et al.*, 1990).

Sequence of PCR products

Plasmid DNA from 15 transformants was prepared (Del Sal *et al.*, 1989). Sequencing data generated from these PCR fragments indicated that 11 out of the 15 represented unique clones. A distinct set of cytochrome P450 consensus amino acids was also found in the translated sequence encoded within the *A. thaliana* PCR inserts. The sequences of the PCR fragments were also compared with those of the petunia OGR-38 F3'H cDNA clone and the snapdragon F3'H cDNA clone. The PCR fragment, p58092.13, was most similar to the F3'H sequences from both petunia and snapdragon.

EXAMPLE 23- Screening of *A. thaliana* cDNA library

To isolate a cDNA clone of the p58092.13 PCR product, an *A. thaliana* ecotype Columbia cDNA library (Newman *et al.*, 1994; D' Alessio *et al.*, 1992) was screened with a ^{32}P-labelled fragment of p58092.13 together with a ^{32}P-labelled fragment of the petunia Ht1 cDNA insert (OGR-38), contained in pCGP1805.

A total of 600,000 pfu was plated at a density of 50,000 pfus per 15 cm diameter plate, as described by D' Alessio *et al* (1992). After phage growth at 37°C plates were stored at 4°C overnight, duplicate lifts were taken onto Colony/Plaque Screen™filters (DuPont) and treated as recommended by the manufacturer.

Prior to hybridization, the duplicate plaque lifts were washed in prewashing solution (50 mM Tris-HCl pH7.5, 1 M NaCl, 1 mM EDTA, 0.1% (w/v) sarcosine) at 65°C for 30 minutes; stripped in 0.4 M sodium hydroxide at 65°C for 30 minutes; then washed in a solution of

0.2 M Tris-HCl pH 8.0, 0.1 x SSC, 0.1% (w/v) SDS at 65°C for 30 minutes and finally rinsed in 2 x SSC, 1.0% (w/v) SDS.

Hybridization conditions included a prehybridization step in 50% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42°C for at least 1 hour. The ^{32}P-labelled fragment of p58092.13 ($2x10^6$cpm/mL) was then added to the hybridization solution and hybridization was continued at 42°C for a further 16 hours. The filters were then washed in 2 x SSC, 1% (w/v) SDS at 42°C for 2 x 1 hour and exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours.

Eleven strongly-hybridizing plaques were picked into PSB and rescreened as detailed above, to isolate purified plaques. These filters were also probed with ^{32}P-labelled fragment of the petunia Ht1 cDNA insert (OGR-38), contained in pCGP1805, under low stringency conditions. Low stringency conditions included prehybridization and hybridization at 42°C in 20% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS and washing in 6xSSC, 1% (w/v) SDS (w/v) at 65°C for 1 hour.

The OGR-38 and p58092.13 probes hybridized with identical plaques. The 11 pure plaques were picked into PSB and the plasmid vectors pZL1 containing the cDNA clones were rescued using the bacterial strain DH10B(Zip). Plasmid DNA was prepared (Del Sal *et al.*, 1989) and the cDNA inserts were released upon digestion with BamHI and EcoRI. The 11 plasmids contained cDNA inserts of between 800bp and 1 kb. Sequence data generated from the 5' region of the cDNA inserts suggested that nine of these clones were identical. Sequence data were generated from the 5' ends of all nine cDNA inserts and the 3' end of only one cDNA insert. The sequence data generated from all clones were compiled to produce the nucleotide and translated sequence shown as SEQ ID NO:7 and SEQ ID NO:8.

The *A. thaliana* putative F3'H sequences were compared with the sequences of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2) and was 64.7% similar to the petunia F3'H cDNA clone, over 745 nucleotides, and 63.7 % similar, over 248 amino

acids.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

Isolation of a F3'H genomic clone from *Arabidopsis thaliana*

To isolate a genomic clone of the *A. thaliana* F3'H gene, a *A. thaliana* ecotype Landsberg erecta genomic DNA library was screened with ^{32}P-labelled p60606.04 fragments. The library was created by cloning partial MboI-digested genomic DNA between BamHI-digested bacteriophage lambda EMBL4 arms. The primary library, which contained 30,000 clones, was amplified once before screening.

The p60606.04 clone, containing a 1 kb fragment of *A. thaliana* F3'H cDNA, was digested with BamHI/EcoRI to excise the insert which was purified using GeneClean (Bio 101). Probe was ^{32}P-labelled using the nick-translation procedure (Sambrook et al., 1989). Approximately 20,000 plaques were probed at high stringency (50% formamide at 37^{o} C) and filters were washed in: 2x SSPE; 2x SSPE, 0.1% (w/v) SDS; 0.1x SSPE, all at 65°C. Re-screening was carried out under the same conditions.

DNA was purified from three positive plaques (λTT7-1, λTT7-5 and λTT7-6) and mapped by digestion with EcoRI and EcoRI/SalI. All three clones had an EcoRI fragment in common. λTT7-1 and λTT7-5 had overlapping but not identical restriction patterns. A Southern blot of these digests was probed as above and, for λTT7-1 and λTT7-5, a common 6.5 kb EcoRI/SalI fragment hybridized. A smaller EcoRI/SalI fragment in λTT7-6 also hybridized and was presumably at the insert boundary.

EcoRI/SalI fragments from lTT7-5 were cloned into pBlueScript SK+ and a clone containing

the 6.5 kb fragment, designated E-5, was identified by hybridization (as above) and insert size. A restriction map was compiled for the fragment using EcoRI, SalI, KpnI, HindIII and BglII in various combinations, and by hybridization to Southern blots of these digests with the BamHI/EcoRI insert from the *A. thaliana* F3'H cDNA clone.

Complete sequence of Tt7 genomic clone

A 6.4 kb BamHI fragment from pTt7-2, containing most of the Tt7 genomic fragment was purified, self-ligated, sonicated, end-repaired, size-fractionated (450bp to 800bp) and cloned into SmaI-cut pUC19 using standard techniques (Sambrook et al., 1989). Recombinant clones were isolated, and plasmid DNA was purified and sequenced using M13-21 or M13 reverse sequencing primers. The sequence from overlapping clones was combined into one contiguous fragment. The sequence of the ends of the Tt7 genomic fragment were also obtained by sequencing with the -21 and REV primers. All of the sequences were combined together to obtain the complete sequence of the 6.5 kb EcoRI/SalI fragment from E-5 (SEQ ID NO:9).

The sequences over the coding region of the arabidopsis Tt7 genomic clone (SEQ ID NO:10, 11, 12 and 13) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and 2). The arabidopsis Tt7 coding region showed 65.4% similarity, over 1066 nucleotides, and 67.1% similarity, over 511 amino acids, to that of the petunia OGR-38 F3'H cDNA clone.

Transformation of a tt7 Arabidopsis mutant

Preparation of binary vector

The EcoRI/SalI fragment from E-5 was cloned into EcoRI/SalI-cut pBI101 (Jefferson *et al.*, 1987). Two separate but identical clones were identified: pBI-Tt7-2 (Figure 15) and pBI-Tt7-4. Both clones were used for transformation of *A. tumefaciens*.

Plant Transformation

Plasmids pBI-Tt7-2, pBI-Tt7-4 and pBI101 were transformed into *Agrobacterium* strain

GV3101 pMP90 by electroporation. Transformants were selected on medium containing 50 μg/mL kanamycin (and 50 μg/mL gentamycin to select for the resident pMP90).

Plasmid DNA, from four transformant colonies for each clone, was isolated and digested with EcoRI/SalI, electrophoresed, Southern blotted, and probed with the Tt7 cDNA insert. For pBI-Tt7-2 and pBI-Tt7-4, the expected insert band was identified.

One transformant for each plasmid (i.e.: one control [pBI101 C4], one each of the two Tt7 clones [pBI-Tt7-2-3 and pBI Tt7-4-4]) was used to vacuum infiltrate the *A. thaliana* tt7 mutant line NW88 (4 pots of 10 plants each for each construct), using the a method essentially as described by Bechtold *et al.* (1993).

Seed from each pot was harvested. One hundred mg of seed (approximately 5,000) was plated on nutrient medium (described by Haughn and Somerville, 1986) containing 50 μg/mL kanamycin. Kanamycin-resistant transformants were visible after 7 to 10 days. In the case of pBI-Tt7-2-3 and pBI-Tt7-4-4, a total of 11 transformants were isolated from 5 different seed lots (i.e.: pots) and all kanamycin-resistant transformants were visibly Tt7 in phenotype and exhibited the characteristic red/purple anthocyanin pigments at the margins of the cotyledons and at the hypocotyl. A single kanamycin-resistant transformant was isolated from only one of the four pots of control transformants and it did not exhibit a "wild-type" Tt7 phenotype.

Complementation of tt7 mutant

These transformants were planted out and grown to maturity and individually harvested for seed. In each case, for pBI-Tt7-2-3 and pBI-Tt7-4-4 transformants, the seeds were visibly more brown than the pale brown seed of the tt7 mutant plants. The seed from the control transformant was indistinguishable from the tt7 mutant parent. These seed were plated out on nutrient medium and nutrient medium with kanamycin added, and scored for the Tt7 phenotype (red/purple anthocyanin pigments at the margins of the cotyledons and at the hypocotyl) and kanamycin resistance. The progeny of at least one transformant for each seed

lot was examined, since these were clearly independent transformation events.

Without exception, kanamycin-resistant seedlings exhibited the Tt7 phenotype while kanamycin-sensitive individuals were tt7. In some cases, kanamycin resistance was weak and variable among a family of seed and it was difficult to unequivocally determine whether individuals were kanamycin resistant or kanamycin sensitive.

EXAMPLE 24- Isolation of a F3'H cDNA clone from *Rosa hybrida*

In order to isolate a Rose F3'H cDNA clone, a *Rosa hybrida* cv. Kardinal petal cDNA library was screened with ^{32}P-labelled fragments of the petunia Ht1 cDNA clone (OGR-38), contained in pCGP1805, and snapdragon F3'H cDNA clone (sdF3'H), contained in pCGP246.

Construction of a petal cDNA library from Rose cv. Kardinal

Total RNA was prepared from the buds of *Rosa hybrida* cv. Kardinal stage 2. At this stage, the tightly closed buds were 1.5 cm high and approximately 0.9 cm wide with pale pink petals.

Frozen tissue (1-3 g) was ground in liquid nitrogen with a mortar and pestle, placed in 25 mL pre-chilled Buffer A [0.2 M boric acid, 10 mM EDTA (sodium salt) (pH 7.6)] and homogenized briefly. The extract was mixed on a rotary shaker until it reached room temperature and an equal volume of phenol/chloroform (1:1 v/v), equilibrated with Buffer A, was added. After mixing for a further 10 minutes, the RNA preparation was centrifuged at 10,000 x *g* for 10 minutes at 20°C. The upper aqueous phase was retained and the phenol interface re-extracted as above. The aqueous phases were pooled and adjusted to 0.1 M sodium acetate (pH 6.0), 2.5 volumes 95% ethanol were added and the mixture was stored at -20°C overnight.

The preparation was centrifuged at 10,000 x *g* for 10 minutes at 4°C, the pellet dissolved

gently in 20 mL Buffer B [25 mM boric acid, 1.25 mM EDTA (sodium salt), 0.1 M NaCl (pH 7.6)] and 0.4 volumes 2-butoxyethanol (2BE) were added. This solution was incubated on ice for 30 minutes. It was then centrifuged at 10,000 x *g* for 10 minutes at 0°C and the supernatant was carefully collected. After addition of 1.0 volume of 2BE and incubation on ice for a further 30 minutes, the supernatant was again centrifuged at 10,000 x *g* for 10 minutes at 0°C. The resulting pellet was gently washed with Buffer A:2BE (1:1 v/v), then with 70% (v/v) ethanol, 0.1 M potassium acetate and finally with 95% ethanol. The pellet was air dried and dissolved in 1 mL diethyl pyrocarbonate (DEPC)-treated water. This was adjusted to 3 M lithium chloride, left on ice for 60 minutes and centrifuged at 10,000 x *g* for 10 minutes at 0°C. The pellet was washed twice with 3 M LiCl and then with 70% ethanol, 0.1 M potassium acetate.

The resulting RNA pellet was dissolved in 400 µL DEPC-treated water and extracted with an equal volume phenol/chloroform. The RNA mix was then centrifuged at 10,000 x *g* for 5 minutes at 20°C, the aqueous phase collected and made to 0.1 M sodium acetate, and a further 2.5 volumes of 95% ethanol were added. After 30 minutes incubation on ice, the mix was centrifuged at 13,000 rpm (5,000 x *g*) for 20 minutes at 20°C and the RNA pellet resuspended gently in 400 µL DEPC-treated water.

Poly $(A)^+$ RNA was selected from the total RNA by Oligotex dT-30 (Takara, Japan) following the manufacturer's protocol. The cDNA was synthesized according to the method in Brugliera *et al.* (1994) and used to construct a non-directional petal cDNA library in the EcoRI site of λZAPII (Stratagene). The total number of recombinants obtained was 3.5×10^5.

After transfecting XL1-Blue cells, the packaged cDNA mixture was plated at 50,000 pfu per 15 cm diameter plate. The plates were incubated at 37°C for 8 hours, and the phage were eluted in 100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl pH 8.0, 0.01% (w/v) gelatin (Phage Storage Buffer (PSB)) (Sambrook *et al.*, 1989). Chloroform was added and the phage stored at 4°C as an amplified library.

200,000 pfus of the amplified library were plated onto NZY plates (Sambrook *et al.*, 1989) at a density of 10,000 pfu per 15 cm plate after transfecting XL1-Blue MRF' cells, and incubated at 37°C for 8 hours. After incubation at 4°C overnight, duplicate lifts (labelled as group A and group B) were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Screening of Kardinal cDNA library for a F3'H cDNA clone

Prior to hybridization, the duplicate plaque lifts were washed in prewashing solution (50 mM Tris-HCl pH7.5, 1 M NaCl, 1 mM EDTA, 0.1% (w/v) sarcosine) at 65°C for 30 minutes; stripped in 0.4 M sodium hydroxide at 65°C for 30 minutes; then washed in a solution of 0.2 M Tris-HCl pH 8.0, 0.1 x SSC, 0.1% (w/v) SDS at 65°C for 30 minutes and finally rinsed in 2 x SSC, 1.0% (w/v) SDS.

The group A filters of the duplicate lifts from the Kardinal cDNA library were screened with ^{32}P-labelled fragments of an NcoI fragment from pCGP1805 containing the petunia Ht1 (OGR-38) cDNA clone, while the group B filters were screened with ^{32}P-labelled fragments of EcoRI/SspI fragment from pCGP246 containing the snapdragon F3'H clone.

Hybridization conditions included a prehybridization step in 10% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42°C for at least 1 hour. The ^{32}P-labelled fragment ($2x10^6$cpm/mL) was then added to the hybridization solution and hybridization was continued at 42°C for a further 16 hours. The filters were then washed at 42°C in 2 x SSC, 1% (w/v) SDS for 2 hours followed by 1 x SSC, 1% (w/v) SDS for 1 hour and finally in 0.2 x SSC/1% (w/v) SDS for 2 hours. The filters were exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours.

Four strongly-hybridizing plaques (R1, R2, R3, R4) were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAP bacteriophage vector were rescued and digested with EcoRI to release the cDNA inserts. Clone R1 contained a 1.0 kb insert while clones R2, R3 and R4 contained inserts of approximately 1.3 kb each. Sequence

data were generated from the 3' and 5' ends of the R4 cDNA insert.

The rose R4 putative F3'H sequence was compared with that of the petunia OGR-38 F3'H sequence. At the nucleotide level, the R4 cDNA clone showed 63.2% and 62.1% similarity over 389 nucleotides at the 5' end and 330 nucleotides at the 3' end, respectively. At the amino acid level, the R4 clone showed 65.4 % and 73.9% similarity over 130 amino acids at the 5' end and 69 amino acids at the 3' end, respectively. Based on the high sequence similarity of the Rose R4 cDNA clone to that of the petunia F3'H cDNA clone (OGR-38), a corresponding "full-length" cDNA clone was isolated, as described in Example 25, below.

EXAMPLE 25- Isolation of a full-length rose F3'H cDNA

In order to isolate a "full-length" F3'H cDNA clone from Rose, the *Rosa hybrida* cv Kardinal petal cDNA library described in Example 24 was screened with ^{32}P-labelled fragments of the rose R4 cDNA clone, described above.

A total of 1.9 x 10^6 pfus of the amplified library were plated onto NZY plates at a density of 100,000 pfus per 15 cm diameter plate after transfecting XL1-Blue MRF' cells, and incubated at 37°C for 8 hours. After incubation at 4°C overnight, duplicate lifts were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Screening of Kardinal cDNA library for full-length F3'H cDNA clones

Prior to hybridization, the duplicate plaque lifts were treated as described in Example 24.

The duplicate lifts from the Kardinal cDNA library were screened with ^{32}P-labelled fragments of an EcoRI fragment from the rose R4 cDNA clone.

Hybridization conditions included a prehybridization step in 50% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42°C for at least 1 hour. The ^{32}P-labelled fragment of the rose R4 cDNA clone (1x10^6cpm/mL) was then added to the

hybridization solution and hybridization was continued at 42°C for a further 16 hours. The filters were then washed in 2 x SSC, 1% (w/v) SDS at 42°C for 2 x 1 hour and exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours.

Seventy-three strongly-hybridizing plaques (1-73) were picked into 1mL of PSB and stored at 4°C overnight. 100μL of each was then aliquoted into a microtitre tray as an ordered array.

XL1-Blue MRF' cells were added to 10mL of molten NZY top agar, poured onto NZY plates (15cm diameter) and allowed to set. A replica plating device was used to transfer the 73 phage isolates in an ordered array onto the NZY plate previously inoculated with the XL1-Blue MRF' cells. After incubation at 37°C for 6 hours followed by 4°C overnight, triplicate lifts (arrays 1, 2 and 3) were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Prior to hybridization, the duplicate plaque lifts were treated as described in Example 24.

The 3 arrays were screened with ^{32}P-labelled fragments of a) an EcoRI/SalI fragment covering the 5' end of the rose R4 cDNA clone, b) an EcoRI/ClaI fragment covering the 5' end of the rose R4 cDNA clone or c) an EcoRI fragment of the entire rose R4 cDNA clone using the hybridisation and washing conditions described above, except that the final wash was in 0.1 x SSC, 0.1% (w/v) SDS at 65°C for 30 minutes. The filters were exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours.

All 73 plaques hybridised with the full R4 cDNA clone (EcoRI fragment) whilst a total of only 17 hybridised with the 5' end of the R4 cDNA clone (either EcoRI/SalI or the EcoRI/ClaI fragments). The 17 phage isolates were rescreened as described above to isolate purified plaques. Pure plaques were obtained from 9 out of the 17 (2, 4, 26, 27, 34, 38, 43, 44, 56). The plasmids contained in the λZAP bacteriophage vector were rescued and the sizes of the cDNA inserts were determined using an EcoRI digestion. The cDNA inserts

ranged from 0.9kb to 1.9kb. Of the nine, only #34 (named pCGP2158) and #38 (named pCGP2159) contained cDNA inserts of approximately 1.9kb. Sequence data were generated from the 3' and 5' ends of the cDNA inserts and showed that clones #34 and #38 represented the same gene.

The complete sequence of the rose cDNA clone (#34) contained in the plasmid pCGP2158 was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook *et al.*, 1989). The sequence (SEQ ID NO:14) contained an open reading frame of 1696 bases which encodes a putative polypeptide of 520 amino acids (SEQ ID NO:15).

The nucleotide and predicted amino acid sequences of the rose F3'H #34 cDNA clone (SEQ ID NO:14 and SEQ ID NO:15) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2) and the snapdragon sdF3'H clone (SEQ ID NO:3 and SEQ ID NO:4). The rose F3'H #34 cDNA clone showed 64.7% similarity ,over 1651 nucleotides, and 72.7% similarity, over 509 amino acids, to that of the petunia OGR-38 cDNA clone, and 67.2% similarity, over 1507 nucleotides, and 68.9 similarity, over 502 amino acids, to that of the snapdragon sdF3'H clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

EXAMPLE 26- Stable expression of the rose F3'H cDNA clone (#34) in petunia petals- Complementation of a ht1/ht1 petunia cultivar

Preparation of pCGP2166

Plasmid pCGP2166 (Figure 16) was constructed by cloning the cDNA insert from pCGP2158 in a "sense" orientation behind the Mac promoter (Comai *et al.*, 1990) of pCGP293 (Brugliera *et al.*, 1994). The plasmid pCGP2158 was digested with EcoRI to release the cDNA insert. The overhanging 5' ends were filled in using DNA polymerase (Klenow fragment) (Sambrook *et al.*, 1989). The cDNA fragment was isolated and ligated with filled in BamHI ends of the pCGP293 binary vector. Correct insertion of the fragment in pCGP2166 was established by restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

The binary vector pCGP2166 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP2166/AGL0 cells were then used to transform Skr4 x SW63 petunia plants (also described in Example 9), to test for stable expression and activity of the enzyme encoded by the gene corresponding to the rose #34 cDNA clone.

EXAMPLE 27- Transgenic plant phenotype analysis

pCGP2166 in Skr4 x SW63

The expression of the introduced rose F3'H cDNA in the Skr4 x SW63 hybrid had a marked effect on flower colour. The stamen tissue of the non-transgenic control is white, whereas the same tissue in most of the transgenic plants was pink. In addition, expression of the rose F3'H cDNA in the Skr4 x SW63 hybrid conferred a dark pink hue (RHSCC# 64C and 74C) to the corolla, which is normally pale lilac (RHSCC# 75C). The colour codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

Acid-hydrolysed floral extracts (see Example 11) were run in a Forestal solvent system (HOAc:water:HCl; 30: 10: 3) (Markham, 1982). The 3' hydroxylated flavonoids, peonidin and quercetin, were readily detected in the petal limbs of the transgenic plants. Only kaempferol and a small amount of malvidin were detected in the non-transgenic Skr4 x SW63

control.

The accumulation of the 3'-hydroxylated anthocyanidin, peonidin and the flavonol, quercetin, in the petals of the transgenic Skr4 x SW63/pCGP2166 plants correlated with the pink and dark pink colours observed in the petals of the same plants.

Preparation of pCGP2169

The binary construct pCGP2169 (Figure 17) was prepared by cloning the cDNA insert from pCGP2158 in a "sense" orientation between the CaMV35S promoter (Franck *et al.*, 1980; Guilley *et al.*, 1982) and *ocs* terminator (De Greve *et al.*, 1982). The plasmid pCGP1634 contained a CaMV35S promoter, β-glucuronidase (GUS) reporter gene encoded by the *E. coli uid*A locus (Jefferson *et al.*, 1987) and *ocs* terminator region in a pUC19 vector. The plasmid pCGP2158 was digested with NcoI/XbaI to release the cDNA insert. The plasmid pCGP1634 was also digested with NcoI/XbaI to release the backbone vector containing the CaMV35S promoter and the *ocs* terminator. The fragments were isolated and ligated together to produce pCGP2167. The plasmid pCGP2167 was subsequently digested with PvuII to release the expression cassette containing the CaMV35S promoter, the rose F3'H cDNA clone and the *ocs* termintor. This expression cassette fragment was isolated and ligated with SmaI ends of pWTT2132 binary vector (DNA Plant Technology Corporation; Oakland, California) to produce pCGP2169 (Figure 17).

The binary vector pCGP2169 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP2169/AGL0 cells are used to transform rose plants, to reduce the amount of 3'-hydroxylated flavonoids.

EXAMPLE 28- Isolation of a putative F3'H cDNA clone from chrysanthemum

In order to isolate a chrysanthemum F3'H cDNA clone, a chrysanthemum cv. Red Minstral petal cDNA library was screened with ^{32}P-labelled fragments of the petunia Ht1 cDNA clone (OGR-38), contained in pCGP1805.

Construction of a petal cDNA library from chrysanthemum cv. Red Minstral

Total RNA was prepared from the petals (stages 3 to 5) of chrysanthemum cv. Red Minstral using Trizol™reagent (Life Technologies) (Chomczynski and Sacchi, 1987) according to the manufacturer's recommendations. Poly(A)+ RNA was enriched from the total RNA, using a mRNA isolation kit (Pharmacia) which relies on oligo-(dT) affinity spun-column chromatography .

A Superscript™cDNA synthesis kit (Life Technologies) was used to construct a petal cDNA library in ZipLox using 5 μg of poly(A)+ RNA isolated from stages 3 to 5 of Red Minstral as template.

30,000 pfus of the library were plated onto LB plates (Sambrook *et al.*, 1989) at a density of 3,000 pfus per 15 cm plate after transfecting Y1090r-, and incubated at 37°C for 16 hours. After incubation at 4°C for one hour, duplicate lifts were taken onto Hybond N+™filters (Amersham) and treated as recommended by the manufacturer.

Screening of the Red Minstral cDNA Library

The duplicate lifts from the Red Minstral petal cDNA library were screened with ^{32}P-labelled fragments of the 1.8 kb Asp718/BamHI insert from pCGP1805.

Hybridization conditions included a prehybridization step in 1mM EDTA (pH8.0), 0.5MNa_2HPO_4 (pH7.2), 7% (w/v) SDS (Church and Gilbert, 1984) at 65°C for at least 1 hour. The ^{32}P-labelled fragments ($1x10^6$cpm/mL) were then added to the hybridization solution and hybridization was continued at 65°C for a further 16 hours. The filters were then washed in 2 x SSC, 0.1% (w/v) SDS at 65°C for 2 x 1 hour and exposed to Kodak BioMax™film with an intensifying screen at -70°C for 48 hours.

Eight strongly-hybridizing plaques were picked into PSB (Sambrook *et al.*, 1989). Of these, 2 (RM6i and RM6ii) were rescreened to isolate purified plaques, using the hybridization conditions as described for the initial screening of the cDNA library. The plasmids

contained in the λZipLox bacteriophage vector were rescued according to the manufacturer's protocol and sequence data was generated from the 3' and 5' ends of the cDNA inserts. The partial sequences of the RM6i and RM6ii cDNA inserts were compared with the complete sequence of the petunia OGR-38 F3'H cDNA clone. The RM6i cDNA clone showed relatively high sequence similarity with that of the petunia OGR-38 cDNA clone, and was further characterised.

The RM6i cDNA insert contained in pCHRM1 was released upon digestion with EcoRI and was approximately 1.68 kb. The complete sequence of RM6i cDNA clone (SEQ ID NO:16) contained in the plasmid pCHRM1 was determined by compilation of sequence from subclones of the RM6i cDNA insert.

The nucleotide and predicted amino acid sequences of the chrysanthemum RM6i cDNA insert (SEQ ID NO:16 and SEQ ID NO:17) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The sequence of the chrysanthemum RM6i cDNA insert showed 68.5% similarity, over 1532 nucleotides, and 73.6 % similarity, over 511 amino acids, to that of the petunia OGR-38 F3'H cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

Construction of pLN85 (antisense binary)

A plasmid designated pLN84 was constructed by cloning the RM6i cDNA insert from pCHRM1 in the "antisense" orientation behind the complete CaMV35S promoter contained in pART7 (Gleave 1992). The plasmid pCHRM1 was digested with NotI to release the cDNA insert. The RM6i cDNA fragment was blunt-ended using T4 DNA polymerase (Sambrook et al., 1989) and purified, following agarose gel electrophoresis and GELase

(Epicentre Technologies). The purified fragment was ligated with SmaI ends of the pART7 shuttle vector to produce pLN84. The plasmid pLN84 was subsequently digested with NotI to release the expression cassette containing CaMV35S: RM6i cDNA: ocs. The expression cassette was isolated as a single fragment and ligated with NotI ends of the pART27 binary vector (Gleave, 1992) to produce pLN85 (Figure 18). Correct insertion of the fragment was established by restriction enzyme analysis of DNA isolated from streptomycin-resistant *E.coli* transformants.

The binary vector pLN85 is introduced into chrysanthemum plants via *Agrobacterium*-mediated transformation, as described in Ledger *et al,* 1991), to reduce the amount of 3'-hydroxylated flavonoids.

EXAMPLE 29- Isolation of a putative F3'H cDNA clone from *Torenia fournieri*

In order to isolate a torenia F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a *Torenia fournieri* cv. Summer Wave petal cDNA library, under low stringency conditions.

Construction of *Torenia fournieri* cv. Summer Wave petal cDNA library

A directional petal cDNA library was prepared from Summer Wave flowers, essentially as described in Example 4.

Screening of Summer Wave petal cDNA library

Lifts of a total of 200,000 of the amplified Summer Wave petal cDNA library were screened with DIG-labelled fragments of the 1.8 kb OGR-38 cDNA insert from pCGP1805. A DIG DNA labelling and detection kit from Boehringer-Mannheim was used according to the manufacturer's recommendations.

Hybridizations were carried out in 30% (v/v) formamide, 5 x SSC, 1% (w/v) SDS at 37°C for 16 hours. The filters were then washed in 5 x SSC, 1% (w/v) SDS at 65°C for 1 hour.

The signals were visualized following the protocol of the DIG DNA labelling and detection kit.

Twelve strongly-hybridizing plaques were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAPII bacteriophage vector were rescued and digested with EcoRI/XhoI to release the cDNA inserts. Most of the twelve clones contained cDNA inserts of approximately 1.8 kb. One clone, THT52, contained the longest 5' non-coding-region sequence. The complete sequence of the torenia cDNA clone (THT52), contained in the plasmid pTHT52, was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook *et al.*, 1989). The sequence (SEQ ID NO:18) contained an open reading frame of 1524 bases which encodes a putative polypeptide of 508 amino acids (SEQ ID NO:19).

The nucleotide and predicted amino acid sequences of the torenia THT52 cDNA clone (SEQ ID NO:18 and SEQ ID NO:19) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The torenia THT52 cDNA clone showed 63.6% similarity, over 1694 nucleotides, and 67.4% similarity, over 515 amino acids, to that of the petunia OGR-38 cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

EXAMPLE 30- The F3'H assay of the torenia THT cDNA clone expressed in yeast

Construction of pYTHT6

The plasmid pYTHT6 (Figure 19) was constructed by cloning the cDNA insert from pTHT6

in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter of pYE22m (Tanaka *et al.*, 1988). The plasmid pTHT6 contained the THT6 cDNA clone. THT6 is identical to THT52, except that its 5' non-coding region is 75 bp shorter .

The 1.7kb THT6 cDNA insert was released from the plasmid pTHT6 upon digestion with EcoRI/XhoI. The THT6 cDNA fragment was isolated, purified and ligated with EcoRI/SalI ends of pYE22m to produce pYTHT6.

Yeast transformation, preparation of yeast extracts and the F3'H assay are described in Example 6.

F3'H activity was detected in extracts of G1315/pYTHT6, but not in extracts of non-transgenic yeast. From this it was concluded that the THT6 cDNA insert contained in pYTHT6, encoded a F3'H.

EXAMPLE 31- Isolation of a putative F3'H cDNA clone from *Pharbitis nil* (Japanese morning glory)

In order to isolate a morning glory F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a Japanese morning glory petal cDNA library, under low stringency conditions.

Construction of Japanese morning glory petal cDNA library

The petal cDNA library from young petals of *Pharbitis nil* (Japanese morning glory) was obtained from Dr Iida (National Institute of Basic Biology, Japan).

Screening of Japanese morning glory petal cDNA library

Lifts of a total of 200,000 of the amplified Japanese morning glory petal cDNA library were screened with DIG-labelled fragments of the 1.8 kb OGR-38 cDNA insert from pCGP1805. A DIG DNA labelling and detection kit from Boehringer-Mannheim was used according to

the manufacturer's recommendations.

Hybridizations were carried out in 30% (v/v) formamide, 5 x SSC, 1% (w/v) SDS at 37°C for 16 hours. The filters were then washed in 5 x SSC, 1% (w/v) SDS at 65°C for 1 hour. The signals were visualized following the protocol of the DIG DNA labelling and detection kit.

Twenty strongly-hybridizing plaques were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAPII bacteriophage vector were rescued and digested with EcoRI/XhoI to release the cDNA inserts. One clone (MHT85) contained a 1.8kb insert. The complete sequence of the Japanese morning glory cDNA clone (MHT85) (SEQ ID NO:20), contained in the plasmid pMHT85, was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook *et al.*, 1989). The MHT85 sequence appears to be 5 bases short of "full-length".

The nucleotide and predicted amino acid sequences of the Japanese morning glory MHT85 cDNA clone (SEQ ID NO:20 and SEQ ID NO:21) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The Japanese morning glory MHT85 cDNA clone showed 69.6% similarity, over 869 nucleotides, and 74.8% similarity, over 515 amino acids, to that of the petunia OGR-38 cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

EXAMPLE 32- Isolation of a putative F3'H cDNA clone from *Gentiana triflora*

In order to isolate a gentian F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a *Gentiana triflora* Pall. var *japonica* Hara petal cDNA library, under low stringency conditions.

Construction of gentian petal cDNA library

A petal cDNA library was prepared from *Gentiana triflora* Pall. var *japonica* Hara flowers, as described by Tanaka *et al.*, 1996.

Screening of gentian petal cDNA library

Lifts of a total of 200,000 of the amplified gentian petal cDNA library were screened with DIG-labelled fragments of the 1.8 kb OGR-38 cDNA insert from pCGP1805. A DIG DNA labelling and detection kit from Boehringer-Mannheim was used according to the manufacturer's recommendations.

Hybridizations were carried out in 30% (v/v) formamide, 5 x SSC, 1% (w/v) SDS at 37°C for 16 hours. The filters were then washed in 5 x SSC, 1% (w/v) SDS at 65°C for 1 hour. The signals were visualized following the protocol of the DIG DNA labelling and detection kit.

Fifteen strongly-hybridizing plaques were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAPII bacteriophage vector were rescued and digested with EcoRI/XhoI to release the cDNA inserts. One clone (GHT13) contained a 1.8kb insert. The sequence of the partial gentian cDNA clone (GHT13) (SEQ ID NO:22), contained in the plasmid pGHT13, was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook *et al.*, 1989).

The nucleotide and predicted amino acid sequences of the gentian GHT13 cDNA clone (SEQ ID NO:22 and SEQ ID NO:23) were compared with those of the petunia OGR-38 F3'H cDNA clone. The gentian GHT13 cDNA clone showed 68.3% similarity, over 1519

nucleotides, and 71.8% similarity, over 475 amino acids, to that of the petunia OGR-38 cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

EXAMPLE 33- Isolation of putative F3'H cDNA clone from lisianthus

In order to isolate a lisianthus F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a lisianthus petal cDNA library, under low stringency conditions.

Construction and screening of lisianthus petal cDNA library

10,000 pfus of a lisianthus petal cDNA library described by Davies *et al.* (1993) and Markham and Offman (1993) were plated onto LB plates (Sambrook *et al.*, 1989) at a density of 3,000 pfus per 15 cm plate after transfecting Y1090r-, and incubated at 37°C for 16 hours. After incubation at 4°C for one hour, duplicate lifts were taken onto Hybond N+™filters (Amersham) and treated as recommended by the manufacturer.

The duplicate lifts from the lisianthus line #54 petal cDNA library were screened with ^{32}P-labelled fragments of the 1.8 kb Asp718/BamHI insert from pCGP1805.

Hybridization conditions included a prehybridization step in 1mM EDTA (pH8.0), 0.5MNa_2HPO_4 (pH7.2), 7% (w/v) SDS (Church and Gilbert, 1984) at 55°C for at least 1 hour. The ^{32}P-labelled fragments ($1x10^6$cpm/mL) were then added to the hybridization solution and hybridization was continued at 55°C for a further 16 hours. The filters were then washed in 2 x SSC, 0.1% (w/v) SDS at 55°C for 2 x 15 minutes, and exposed to Kodak

BioMax™film with an intensifying screen at -70°C for 18 hours.

Twelve strongly-hybridizing plaques were picked into PSB (Sambrook *et al.*, 1989) and rescreened to isolate purified plaques, using the hybridization conditions as described for the initial screening of the cDNA library. Sequence data were generated from the 3' and 5' ends of the cDNA inserts of four clones.

Based on sequence comparisons, pL3-6 showed similarity with the petunia OGR-38 F3'H cDNA clone and was further characterised.

The 2.2 kb cDNA insert, contained in pL3-6, was subsequently found to contain 3 truncated cDNA clones, the longest (L3-6) having high sequence similarity to the petunia OGR-38 cDNA sequence. The sequence of this L3-6 partial cDNA clone contained in the plasmid pL3-6 was determined by compilation of sequence from subclones of the L3-6 cDNA insert (SEQ ID NO:24).

The nucleotide and predicted amino acid sequences of the lisianthus L3-6 cDNA clone (SEQ ID NO:24 and SEQ ID NO:25) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The sequence of the lisianthus L3-6 cDNA clone showed 71.4% similarity, over 1087 nucleotides, and 74.6% similarity, over 362 amino acids, to that of the petunia OGR-38 F3'H cDNA clone .

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

Further investigation of the remaining clones isolated from the screening of the lisianthus

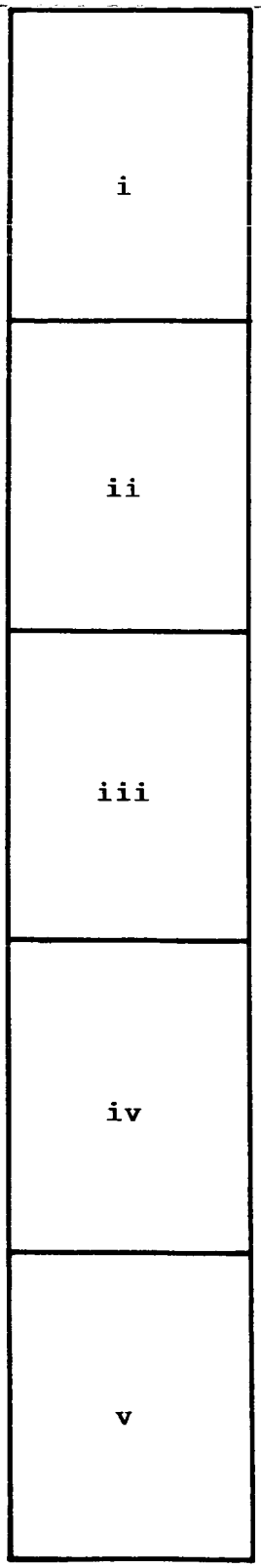
library identified another putative F3'H cDNA clone (L3-10), contained in the plasmid pL3-10. The L3-10 cDNA insert is approximately 1.8kb and appears to represent a "full-length" clone.

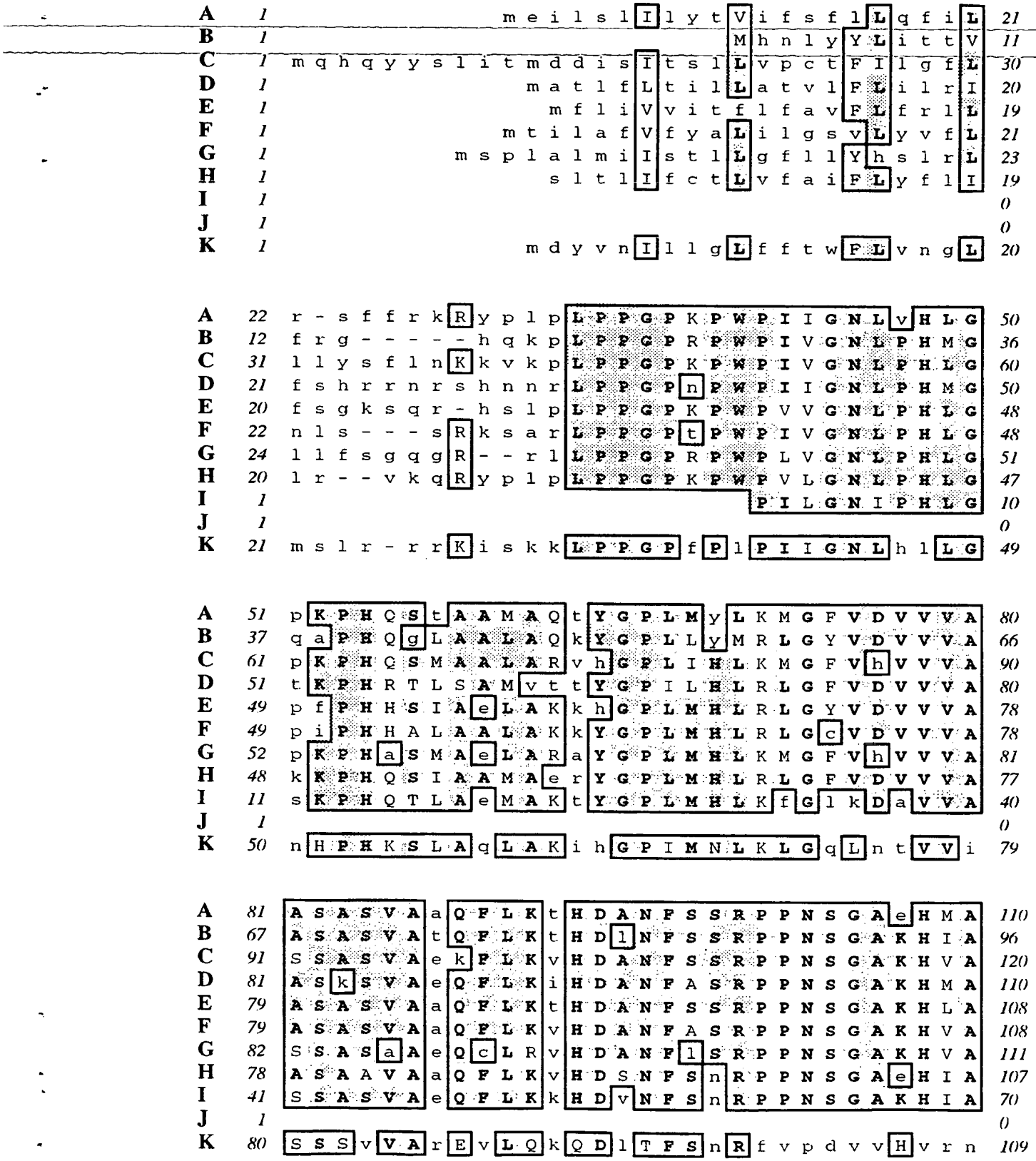
EXAMPLE 34-Alignments and comparisons among nucleotide and amino acid sequences disclosed herein

Multiple sequence alignments were performed using the ClustalW program as described in Example 3. Table 7 (below) provides a multiple sequence alignment of the predicted amino acid sequences of petunia OGR-38 (A); carnation (B); snapdragon (C); arabidopsis Tt7 coding region (D); rose (E) chrysanthemum (F); torenia (G); morning glory (H); gentian (partial sequence) (I); lisianthus (partial sequence) (J) and the petunia 651 cDNA (K). Conserved amino acids are shown in bolded capital letters and are boxed and shaded. Similar amino acids are shown in capital letters and are only lightly shaded, and dissimilar amino acids are shown in lower case letters.

Nucleotide and amino acid sequences of the F3'H cDNA clones from the above mentioned species and the coding region of the genomic clone from arabidopsis were compared using the LFASTA program, as described in Example 3. Summaries of similarity comparisons are presented in Tables 8 to 12, below.

TABLE 7

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B 1 MhnlyYLittV 11
C 1 mqhqyyslitmddisItslLvpctFIlgfL 30
D 1 matlfLtilLatvlFLilrI 20
E 1 mflivvitflfavFLflrlL 19
F 1 mtilafVfyaLilgsvLyvfL 21
G 1 msplalmiIstlLgfllYhslrL 23
H 1 sltlIfctLvfaiFLyflI 19
I 1 0
J 1 0
K 1 mdyvnIllgLfftwFLvngL 20
A 22 r-sffrkRypl pLPPGPKPWPIIGNLvHLG 50
B 12 frg-----hqkp LPPGPRPWPIVGNLPHMG 36
C 31 llysflnKkvkp LPPGPKPWPIVGNLPHLG 60
D 21 fshrrnrshnnr LPPGPnPWPIIGNLPHMG 50
E 20 fsgksqr-hslp LPPGPKPWPVVGNLPHLG 48
F 22 nls---sRksar LPPGPtPWPIVGNLPHLG 48
G 24 llfsgqgR--rl LPPGPRPWPLVGNLPHLG 51
H 20 lr--vkqRypl pLPPGPKPWPVLGNLPHLG 47
I 1 PILGNIPHLG 10
J 1 0
K 21 mslr-rrKisk kLPPGPfPlPIIGNLhlLG 49
A 51 pKPHQStAAMAQtYGPLMyLKMGFVDVVVA 80
B 37 qaPHQgLAALAQkYGPLLyMRLGYVDVVVA 66
C 61 pKPHQSMAALARvhGPLIHLKMGFVhVVVA 90
D 51 tKPHRTLSAMvttYGPILHLRLGFVDVVVA 80
E 49 pfPHHSIAeLAKkhGPLMHLRLGYVDVVVA 78
F 49 piPHHALAALAKkYGPLMHLRLGcVDVVVA 78
G 52 pKPHaSMAeLARaYGPLMHLKMGFVhVVVA 81
H 48 kKPHQSIAAMAerYGPLMHLRLGFVDVVVA 77
I 11 sKPHQTLAeMAKtYGPLMHLKfGlkDaVVA 40
J 1 0
K 50 nHPHKSLAqLAKih GPIMNLKLGqLntVVi 79
A 81 ASASVAaQFLKtHDANFSSRPPNSGAeHMA 110
B 67 ASASVAtQFLKtHDlNFSSRPPNSGAKHIA 96
C 91 SSASVAekFLKvHDANFSSRPPNSGAKHVA 120
D 81 ASkSVAeQFLKiHDANFASRPPNSGAKHMA 110
E 79 ASASVAaQFLKtHDANFSSRPPNSGAKHLA 108
F 79 ASASVAaQFLKvHDANFASRPPNSGAKHVA 108
G 82 SSASaAeQcLRvHDANFlSRPPNSGAKHVA 111
H 78 ASAAVAaQFLKvHDSNFSnRPPNSGAeHIA 107
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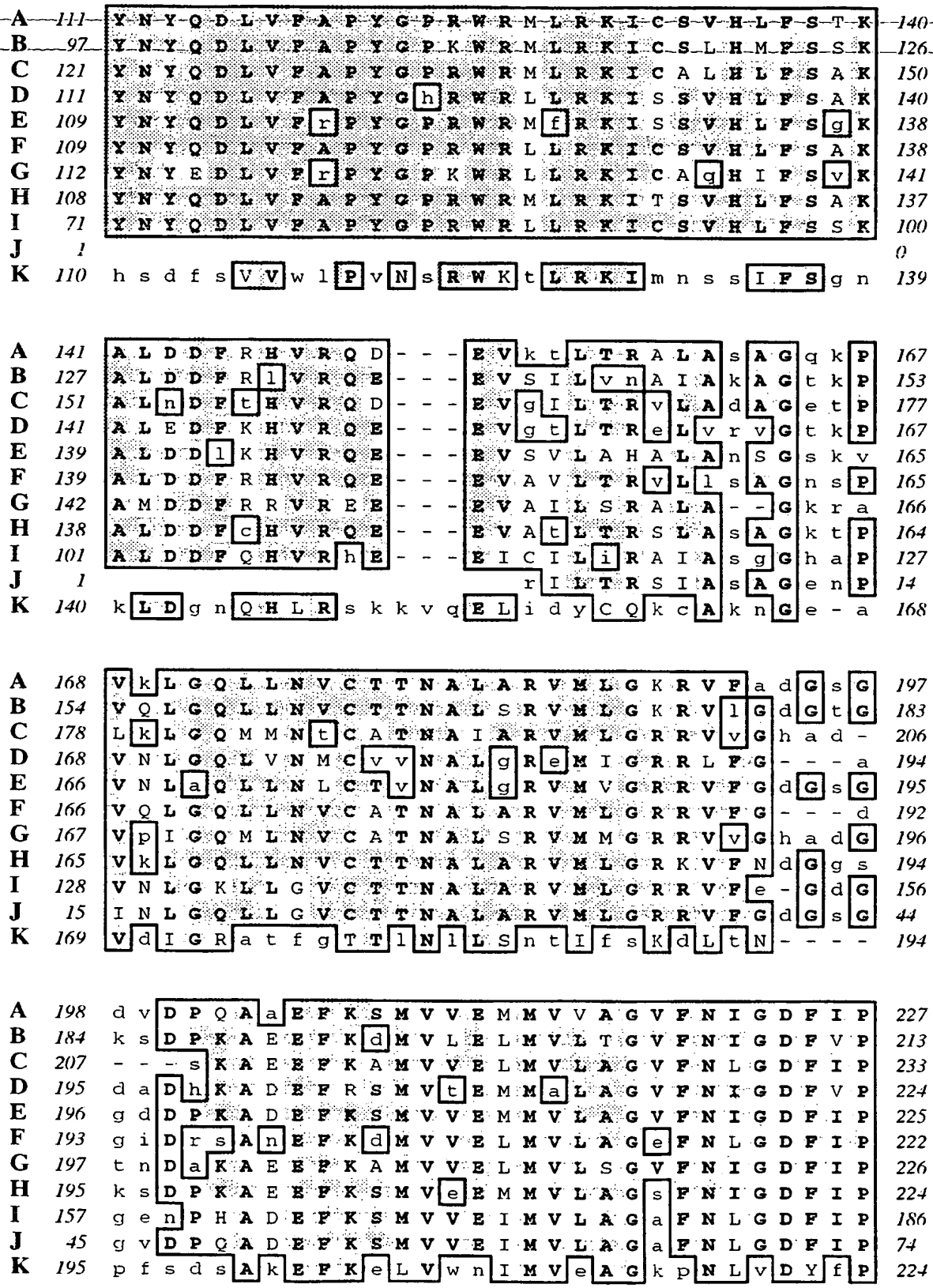
J 1 0
K 80 SSSvVArEvLQkQDlTFSnRfvpdvvHvrn 109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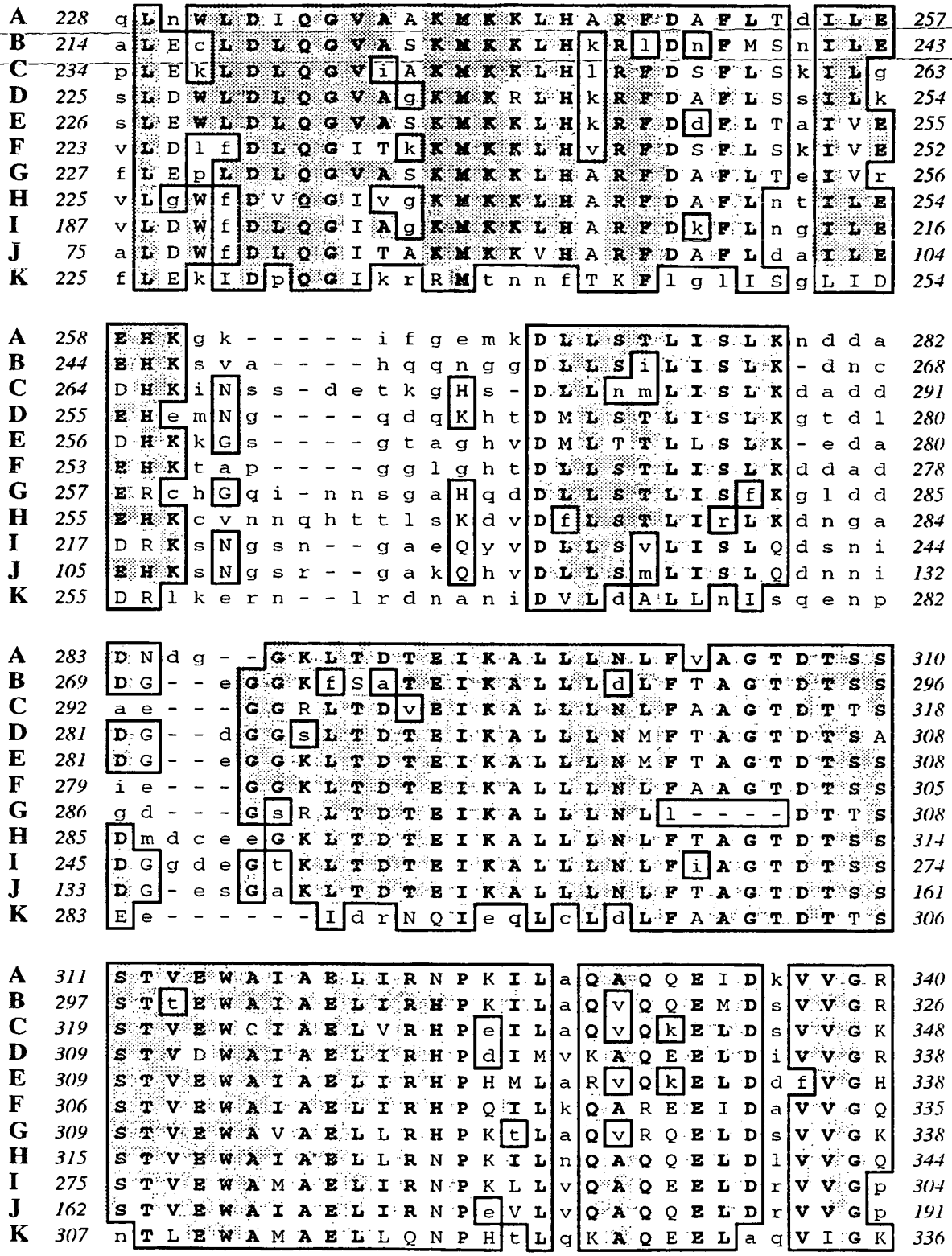
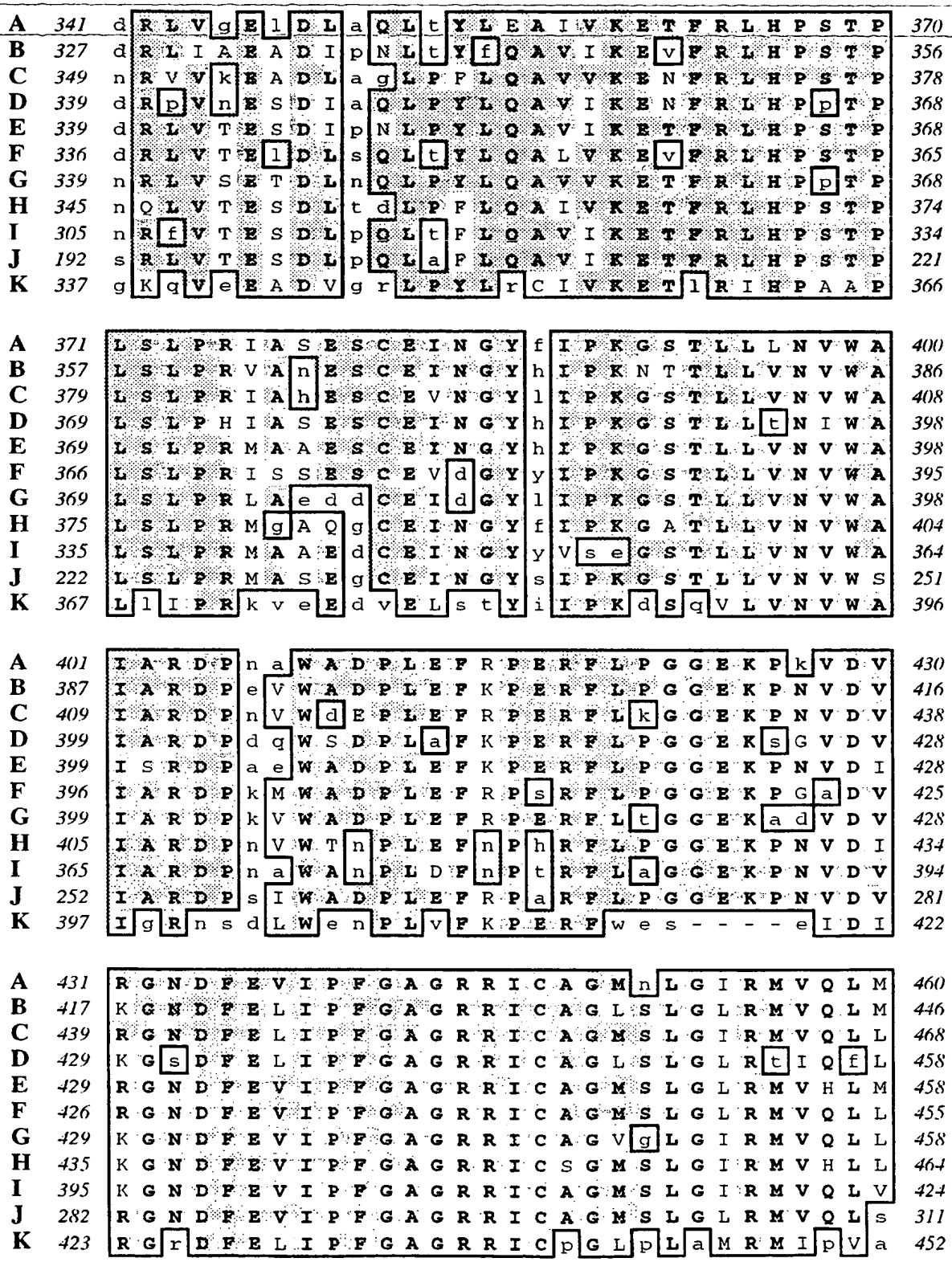
B 97 Y N Y Q D L V F A P Y G P K W R M L R K I C S L H M F S S K 126
C 121 Y N Y Q D L V F A P Y G P R W R M L R K I C A L H L F S A K 150
D 111 Y N Y Q D L V F A P Y G h R W R L L R K I S S V H L F S A K 140
E 109 Y N Y Q D L V F r P Y G P R W R M f R K I S S V H L F S g K 138
F 109 Y N Y Q D L V F A P Y G P R W R L L R K I C S V H L F S A K 138
G 112 Y N Y E D L V F r P Y G P K W R L L R K I C A g H I F S v K 141
H 108 Y N Y Q D L V F A P Y G P R W R M L R K I T S V H L F S A K 137
I 71 Y N Y Q D L V F A P Y G P R W R L L R K T C S V H L F S S K 100
J 1 0
K 110 h s d f s V V w l P v N s R W K t L R K I m n s s I F S g n 139
A 141 A L D D F R H V R Q D - - - E V k t L T R A L A s A G q k P 167
B 127 A L D D F R l V R Q E - - - E V S I L v n A I A k A G t k P 153
C 151 A L n D F t H V R Q D - - - E V g I L T R v L A d A G e t P 177
D 141 A L E D F K H V R Q E - - - E V g t L T R e L v r v G t k P 167
E 139 A L D D l K H V R Q E - - - E V S V L A H A L A n S G s k v 165
F 139 A L D D F R H V R Q E - - - E V A V L T R v L l s A G n s P 165
G 142 A M D D F R R V R E E - - - E V A I L S R A L A - - G k r a 166
H 138 A L D D F c H V R Q E - - - E V A t L T R S L A s A G k t P 164
I 101 A L D D F Q H V R h E - - - E I C I L i R A I A s g G h a P 127
J 1 r I L T R S I A s A G e n P 14
K 140 k L D g n Q H L R s k k v q E L i d y C Q k c A k n G e - a 168
A 168 V k L G Q L L N V C T T N A L A R V M L G K R V F a d G s G 197
B 154 V Q L G Q L L N V C T T N A L S R V M L G K R V l G d G t G 183
C 178 L k L G Q M M N t C A T N A I A R V M L G R R V v G h a d - 206
D 168 V N L G Q L V N M C v v N A L g R e M I G R R L F G - - - a 194
E 166 V N L a Q L L N L C T v N A L g R V M V G R R V F G d G s G 195
F 166 V Q L G Q L L N V C A T N A L A R V M L G R R V F G - - - d 192
G 167 V p I G Q M L N V C A T N A L S R V M M G R R V v G h a d G 196
H 165 V k L G Q L L N V C T T N A L A R V M L G R K V F N d G g s 194
I 128 V N L G K L L G V C T T N A L A R V M L G R R V F e - G d G 156
J 15 I N L G Q L L G V C T T N A L A R V M L G R R V F G d G s G 44
K 169 V d I G R a t f g T T l N l L S n t I f s K d L t N - - - - 194
A 198 d v D P Q A a E F K S M V V E M M V V A G V F N I G D F I P 227
B 184 k s D P K A E E F K d M V L E L M V L T G V F N I G D F V P 213
C 207 - - - s K A E E F K A M V V E L M V L A G V F N L G D F I P 233
D 195 d a D h K A D E F R S M V t E M M a L A G V F N I G D F V P 224
E 196 g d D P K A D E F K S M V V E M M V L A G V F N I G D F I P 225
F 193 g i D r s A n E F K d M V V E L M V L A G e F N L G D F I P 222
G 197 t n D a K A E E F K A M V V E L M V L S G V F N I G D F I P 226
H 195 k s D P K A E E F K S M V e E M M V L A G s F N I G D F I P 224
I 157 g e n P H A D E F K S M V V E I M V L A G a F N L G D F I P 186
J 45 g v D P Q A D E F K S M V V E I M V L A G a F N L G D F I P 74
K 195 p f s d s A k E F K e L V w n I M V e A G k p N L v D Y f P 224

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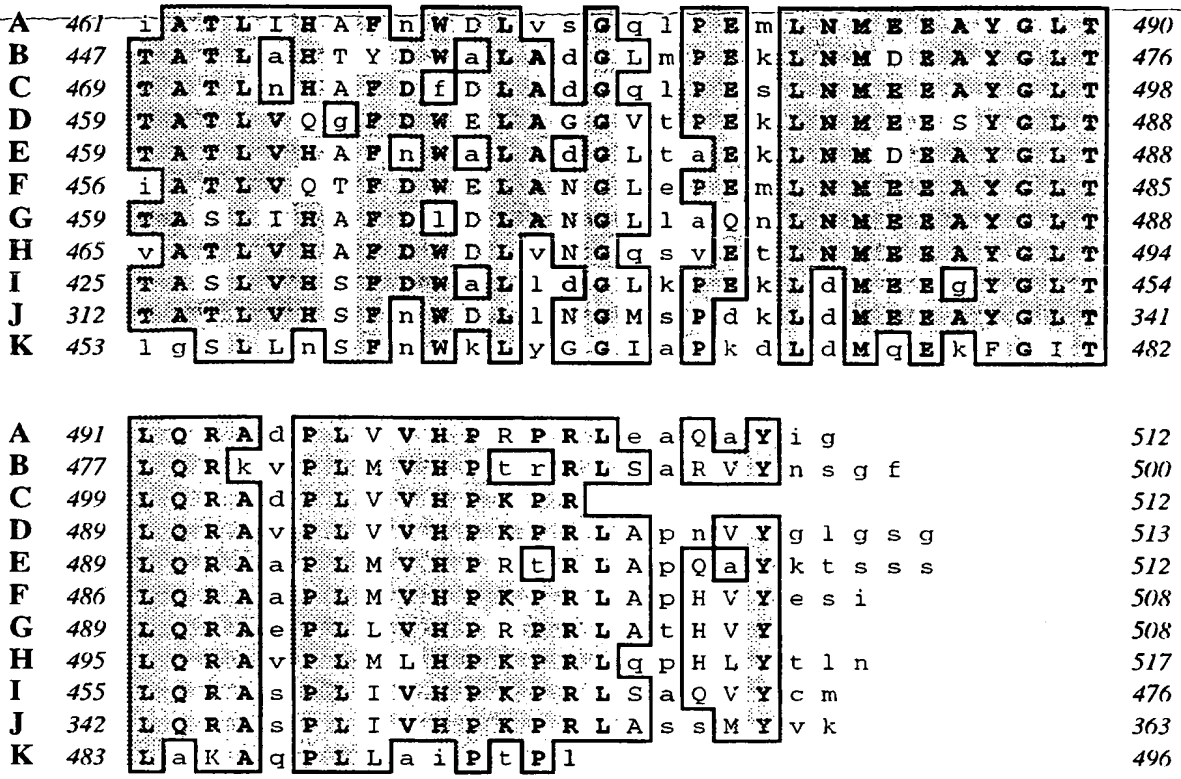
B 327 d R L I A E A D I p N L t Y f Q A V I K E v F R L H P S T P 356
C 349 n R V V k E A D L a g L P P L Q A V V K E N F R L H P S T P 378
D 339 d R p V n E S D I a Q L P Y L Q A V I K E N F R L H P p T P 368
E 339 d R L V T E S D I p N L P Y L Q A V I K E T F R L H P S T P 368
F 336 d R L V T E l D L s Q L t Y L Q A L V K E v F R L H P S T P 365
G 339 n R L V S E T D L n Q L P Y L Q A V V K E T F R L H P p T P 368
H 345 n Q L V T E S D L t d L P F L Q A I V K E T F R L H P S T P 374
I 305 n R f V T E S D L p Q L t F L Q A V I K E T F R L H P S T P 334
J 192 s R L V T E S D L p Q L a F L Q A V I K E T F R L H P S T P 221
K 337 g K q V e E A D V g r L P Y L r C I V K E T l R I H P A A P 366
A 371 L S L P R I A S E S C E I N G Y f I P K G S T L L L N V W A 400
B 357 L S L P R V A n E S C E I N G Y h I P K N T T L L V N V W A 386
C 379 L S L P R I A h E S C E V N G Y l I P K G S T L L V N V W A 408
D 369 L S L P H I A S E S C E I N G Y h I P K G S T L L t N I W A 398
E 369 L S L P R M A A E S C E I N G Y h I P K G S T L L V N V W A 398
F 366 L S L P R I S S E S C E V d G Y y I P K G S T L L V N V W A 395
G 369 L S L P R L A e d d C E I d G Y l I P K G S T L L V N V W A 398
H 375 L S L P R M g A Q g C E I N G Y f I P K G A T L L V N V W A 404
I 335 L S L P R M A A E d C E I N G Y y V s e G S T L L V N V W A 364
J 222 L S L P R M A S E g C E I N G Y s I P K G S T L L V N V W S 251
K 367 L l I P R k v e E d v E L s t Y i I P K d S q V L V N V W A 396
A 401 I A R D P n a W A D P L E F R P E R F L P G G E K P k V D V 430
B 387 I A R D P e V W A D P L E F K P E R F L P G G E K P N V D V 416
C 409 I A R D P n V W d E P L E F R P E R F L k G G E K P N V D V 438
D 399 I A R D P d q W S D P L a F K P E R F L P G G E K s G V D V 428
E 399 I S R D P a e W A D P L E F K P E R F L P G G E K P N V D I 428
F 396 I A R D P k M W A D P L E F R P s R F L P G G E K P G a D V 425
G 399 I A R D P k V W A D P L E F R P E R F L t G G E K a d V D V 428
H 405 I A R D P n V W T n P L E F n P h R F L P G G E K P N V D I 434
I 365 I A R D P n a W A n P L D F n P t R F L a G G E K P N V D V 394
J 252 I A R D P s I W A D P L E F R P a R F L P G G E K P N V D V 281
K 397 I g R n s d L W e n P L v F K P E R F w e s - - - - e I D I 422
A 431 R G N D F E V I P F G A G R R I C A G M n L G I R M V Q L M 460
B 417 K G N D F E L I P F G A G R R I C A G L S L G L R M V Q L M 446
C 439 R G N D F E L I P F G A G R R I C A G M S L G I R M V Q L L 468
D 429 K G s D F E L I P F G A G R R I C A G L S L G L R t I Q f L 458
E 429 R G N D F E V I P F G A G R R I C A G M S L G L R M V H L M 458
F 426 R G N D F E V I P F G A G R R I C A G M S L G L R M V Q L L 455
G 429 K G N D F E V I P F G A G R R I C A G V g L G I R M V Q L L 458
H 435 K G N D F E V I P F G A G R R I C S G M S L G I R M V H L L 464
I 395 K G N D F E V I P F G A G R R I C A G M S L G I R M V Q L V 424
J 282 R G N D F E V I P F G A G R R I C A G M S L G L R M V Q L s 311
K 423 R G r D F E L I P F G A G R R I C p G L p L a M R M I p V a 452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B 447 T A T L a H T Y D W a L A d G L m P E k L N M D E A Y G L T 476
C 469 T A T L n H A F D f D L A d G q l P E s L N M E E A Y G L T 498
D 459 T A T L V Q g P D W E L A G G V t P E k L N M E E S Y G L T 488
E 459 T A T L V H A F n W a L A d G L t a E k L N M D E A Y G L T 488
F 456 i A T L V Q T F D W E L A N G L e P E m L N M E E A Y G L T 485
G 459 T A S L I H A F D l D L A N G L l a Q n L N M E E A Y G L T 488
H 465 v A T L V H A F D W D L v N G q s v E t L N M E E A Y G L T 494
I 425 T A S L V H S F D W a L l d G L k P E k L d M E E g Y G L T 454
J 312 T A T L V H S F n W D L l N G M s P d k L d M E E A Y G L T 341
K 453 l g S L L n S F n W k L y G G I a P k d L d M q E k F G I T 482
A 491 L Q R A d P L V V H P R P R L e a Q a Y i g 512
B 477 L Q R k v P L M V H P t r R L S a R V Y n s g f 500
C 499 L Q R A d P L V V H P K P R 512
D 489 L Q R A v P L V V H P K P R L A p n V Y g l g s g 513
E 489 L Q R A a P L M V H P R t R L A p Q a Y k t s s s 512
F 486 L Q R A a P L M V H P K P R L A p H V Y e s i 508
G 489 L Q R A e P L L V H P R P R L A t H V Y 508
H 495 L Q R A v P L M L H P K P R L q p H L Y t l n 517
I 455 L Q R A s P L I V H P K P R L S a Q V Y c m 476
J 342 L Q R A s P L I V H P K P R L A s s M Y v k 363
K 483 L a K A q P L L a i P t P l 496

TABLE 8

Percentage of sequence similarity between F3'H sequence of petunia OGR-38 and F3'H sequences from other species and other P450 molecules

Species / Clone	Number of nucleotides (nt)	Number of amino acids (aa)	%similarity to OGR-38 / no. nt (area of similarity)	%similarity to OGR-38 / no. aa (area of similarity)
Petunia OGR-38	**1789nt**	**512aa**		
Snapdragon F3'H cDNA	1711nt	512aa	69.0% /1573nt (19-1578)	72.2% /507aa (1-504)
Arabidopsis partial F3'H cDNA	971nt	270aa	64.7% /745nt (854-1583)	63.7% /248aa (269-510)
Arabidopsis Tt7 coding region	1774nt	513aa	65.4% /1066nt	67.1%/511aa
Carnation F3'H cDNA	1745nt	496aa	67.3% /1555nt (28-1571)	71.5%/488aa (17-503)
Rose F3'H cDNA	1748nt	513aa	64.7% /1651nt (56-1699)	72.7%/509aa (7-510)
Gentian partial F3'H cDNA	1667nt	476aa	68.3%/1519nt (170-1673)	71.8%/475aa (40-510)
Morning Glory F3'H cDNA	1824nt	517aa	69.6%/869nt (60-1000)	74.8%/515aa (3-510)
Chrysanthemum F3'H cDNA	1660nt	508aa	68.5%/1532nt (50-1580)	73.6%/511aa (1-510)
Lisianthus partial F3'H cDNA	1214nt	363aa	71.4%/1087nt (520-1590)	74.6%/362aa (160-510)
Torenia F3'H cDNA	1815nt	508aa	63.6%/1694nt (90-1780)	67.4%/515aa (1-510)
Petunia Hf1 cDNA	1812nt	508aa	58.9% /1471nt (29-1474)	49.9% /513aa (1-511)
Petunia Hf2 cDNA	1741nt	508aa	58.9% /1481nt (37-1498)	49.1%/511aa (3-510)
Petunia 651 cDNA	1716nt	496aa	53.5% /1284nt (50-1309)	38.0% /502aa (7-503)
Mung Bean C4H cDNA	1766nt	505aa	56.0% /725nt (703-1406)	29.2% /511aa (1-503)

TABLE 9

Percentage of sequence similarity between F3'H sequence of Snapdragon and F3'H sequences from other species and other P450 molecules

Species / Clone	Number of nucleotides (nt)	Number of amino acids (aa)	%similarity to snapdragon/no. nt	%similarity to snapdragon/no. aa
Snapdragon	**1711nt**	**512aa**		
Petunia OGR-38 F3'H cDNA	1789nt	512aa	69.0% /1573nt	72.2% /507aa
Arabidopsis partial F3'H cDNA	971nt	270aa	64.5% /740nt	60.4% /240aa
Carnation F3'H cDNA	1745nt	496aa	66.7% /1455nt	68.4%/487aa
Torenia F3'H cDNA	1815nt	508aa	67.6%/1603nt	70.3%/505aa
Rose F3'H cDNA	1748nt	513aa	67.2%/1507nt	68.9%/502aa
Petunia Hf1 cDNA	1812nt	508aa	57.3% /1563nt	49.3%/491aa
Petunia Hf2 cDNA	1741nt	508aa	57.7% /1488nt	47.8%/508aa
Petunia 651 cDNA	1716nt	496aa	54.4% /1527nt	39.0% /493aa
Mung Bean C4H cDNA	1766nt	505aa	50.6% /1344nt	32.0% /490aa

TABLE 10

Percentage of sequence similarity between F3'H sequence of Arabidopsis and F3'H sequences from other species and other P450 molecules

Species / Clone	Number of nucleotides (nt)	Number of amino acids (aa)	%similarity to Arabidopsis/no. nt	%similarity to Arabidopsis/no. aa
Arabidopsis	**971nt**	**270aa**		
Petunia OGR-38 F3'H cDNA	1789nt	512aa	64.7% /745nt	63.7% /248aa
Snapdragon F3'H cDNA	1711nt	512aa	64.5%/740nt	60.4%/240aa
Carnation F3'H cDNA	1745nt	496aa	64.7% /782nt	60.6%/241aa
Rose F3'H cDNA	1748nt	513aa	68.5%/739nt	63.7%/248aa
Petunia 651 cDNA	1716nt	496aa	57.0%/521nt	40.5%/227aa
Petunia Hf1 cDNA	1812nt	508aa	58.2% /632nt	46.5% /243aa
Petunia Hf2 cDNA	1741nt	508aa	57.4% /632nt	46.1%/243aa

TABLE 11

Percentage of sequence similarity between F3'H sequence of Rose and F3'H sequences from other species and other P450 molecules

Species / Clone	Number of nucleotides (nt)	Number of amino acids (aa)	%similarity to Rose / no. nt	%similarity to Rose / no. aa
Rose	**1748bp**	**513aa**		
Petunia OGR-38 F3'H cDNA	1789bp	512aa	64.7% /1651nt	72.7%/509aa
Snapdragon F3'H cDNA	1711bp	512aa	67.2%/1507	68.9%/502aa
Carnation F3'H cDNA	1745bp	496aa	67.4%/1517nt	72.6%/486aa
Arabidopsis partial F3'H cDNA	971bp	270aa	68.5%/739nt	63.7%/248aa
Petunia 651 cDNA	1716bp	496aa	53.1%/1182nt	37.8%/502aa
Petunia Hf1 cDNA	1812bp	506aa	57%/1366nt	49.9%/503aa
Petunia Hf2 cDNA	1741bp	508aa	57.3%/1331nt	49.1%/505aa
Mung Bean C4H cDNA	1766bp	505aa	52.4%/1502nt	32.0%/510aa

TABLE 12

Percentage of sequence similarity between coding region of Arabidopsis tt7 genomic sequence and F3'H cDNA sequences from other species and other P450 molecules

Species / Clone	Number of nucleotides (nt)	Number of amino acids (aa)	%similarity to Arabidopsis tt7 / no. nt	%similarity to Arabidopsis tt7 / no. aa
Arabidopsis Tt7 coding region	**1774nt**	**513aa**		
Petunia OGR-38 F3'H cDNA	1789nt	512aa	65.4% /1066nt	67.1%/511aa
Snapdragon F3'H cDNA	1711nt	512aa	62.7%/990nt	64.9%/504aa
Carnation F3'H cDNA	1745nt	496aa	63.2%/1050nt	65.9%/495aa
Rose F3'H cDNA	1748nt	513aa	65.5%/1076nt	68%/512aa
Petunia 651 cDNA	1716nt	496aa	56.5%/990nt	36.5%/502aa
Petunia Hf1 F3'H cDNA	1812nt	506aa	56.8%/995nt	47.5%/509aa
Petunia Hf2 F3'H cDNA	1741nt	508aa	55.2%/1063nt	46.8%/509aa

Those skilled in the art, will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (OTHER THAN US): FLORIGENE LIMITED
(US ONLY): Filippa BRUGLIERA, Timothy Albert HOLTON, Michael Zenon MICHAEL

(ii) TITLE OF INVENTION: GENETIC SEQUENCES ENCODING FLAVONOID PATHWAY ENZYMES AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 40

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:

(B) FILING DATE: 28-FEB-1997

(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PN8386

(B) FILING DATE: 28-FEB-1997

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES, DR E JOHN L

(C) REFERENCE/DOCKET NUMBER: EJH/AF

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777

(B) TELEFAX: +61 3 9254 2770

(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1789 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 50..1586

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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                                                        Met Glu
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ATC TTA AGC CTA ATT CTG TAC ACC GTC ATT TTC TCA TTT CTT CTA CAA        103
Ile Leu Ser Leu Ile Leu Tyr Thr Val Ile Phe Ser Phe Leu Leu Gln
          5                  10                  15

TTC ATT CTT AGA TCA TTT TTC CGT AAA CGT TAC CCT TTA CCA TTA CCA        151
Phe Ile Leu Arg Ser Phe Phe Arg Lys Arg Tyr Pro Leu Pro Leu Pro
     20                  25                  30

CCA GGT CCA AAA CCA TGG CCA ATT ATA GGA AAC CTA GTC CAT CTT GGA        199
Pro Gly Pro Lys Pro Trp Pro Ile Ile Gly Asn Leu Val His Leu Gly
 35                  40                  45                  50

CCC AAA CCA CAT CAA TCA ACT GCA GCC ATG GCT CAA ACT TAT GGA CCA        247
Pro Lys Pro His Gln Ser Thr Ala Ala Met Ala Gln Thr Tyr Gly Pro
                 55                  60                  65
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CTC ATG TAT CTT AAG ATG GGG TTC GTA GAC GTG GTG GTT GCA GCC TCG      295
Leu Met Tyr Leu Lys Met Gly Phe Val Asp Val Val Val Ala Ala Ser
                70                  75                  80

GCA TCG GTT GCA GCT CAG TTC TTG AAA ACT CAT GAT GCT AAT TTC TCG      343
Ala Ser Val Ala Ala Gln Phe Leu Lys Thr His Asp Ala Asn Phe Ser
            85                  90                  95

AGC CGT CCA CCA AAT TCT GGT GCA GAA CAT ATG GCT TAT AAT TAT CAG      391
Ser Arg Pro Pro Asn Ser Gly Ala Glu His Met Ala Tyr Asn Tyr Gln
        100                 105                 110

GAT CTT GTT TTT GCA CCT TAT GGA CCT AGA TGG CGT ATG CTT AGG AAA      439
Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys
115                 120                 125                 130

ATT TGC TCA GTT CAC CTT TTC TCT ACC AAG GCT TTA GAT GAC TTC CGC      487
Ile Cys Ser Val His Leu Phe Ser Thr Lys Ala Leu Asp Asp Phe Arg
                135                 140                 145

CAT GTC CGC CAG GAT GAA GTG AAA ACA CTG ACG CGC GCA CTA GCA AGT      535
His Val Arg Gln Asp Glu Val Lys Thr Leu Thr Arg Ala Leu Ala Ser
            150                 155                 160

GCA GGC CAA AAG CCA GTC AAA TTA GGT CAG TTA TTG AAC GTG TGC ACG      583
Ala Gly Gln Lys Pro Val Lys Leu Gly Gln Leu Leu Asn Val Cys Thr
        165                 170                 175

ACG AAC GCA CTC GCG CGA GTA ATG CTA GGT AAG CGA GTA TTT GCC GAC      631
Thr Asn Ala Leu Ala Arg Val Met Leu Gly Lys Arg Val Phe Ala Asp
    180                 185                 190

GGA AGT GGC GAT GTT GAT CCA CAA GCG GCG GAG TTC AAG TCA ATG GTG      679
Gly Ser Gly Asp Val Asp Pro Gln Ala Ala Glu Phe Lys Ser Met Val
195                 200                 205                 210

GTG GAA ATG ATG GTA GTC GCC GGT GTT TTT AAC ATT GGT GAT TTT ATT      727
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Val Glu Met Met Val Val Ala Gly Val Phe Asn Ile Gly Asp Phe Ile
                215                 220                 225

CCG CAA CTT AAT TGG TTA GAT ATT CAA GGT GTA GCC GCT AAA ATG AAG      775
Pro Gln Leu Asn Trp Leu Asp Ile Gln Gly Val Ala Ala Lys Met Lys
            230                 235                 240

AAG CTC CAC GCG CGT TTC GAC GCG TTC TTG ACT GAT ATA CTT GAA GAG      823
Lys Leu His Ala Arg Phe Asp Ala Phe Leu Thr Asp Ile Leu Glu Glu
        245                 250                 255

CAT AAG GGT AAA ATT TTT GGA GAA ATG AAA GAT TTG TTG AGT ACT TTG      871
His Lys Gly Lys Ile Phe Gly Glu Met Lys Asp Leu Leu Ser Thr Leu
    260                 265                 270

ATC TCT CTT AAA AAT GAT GAT GCG GAT AAT GAT GGA GGG AAA CTC ACT      919
Ile Ser Leu Lys Asn Asp Asp Ala Asp Asn Asp Gly Gly Lys Leu Thr
275                 280                 285                 290

GAT ACA GAA ATT AAA GCA TTA CTT TTG AAC TTG TTT GTA GCT GGA ACA      967
Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Val Ala Gly Thr
                295                 300                 305

GAC ACA TCT TCT AGT ACA GTT GAA TGG GCC ATT GCT GAG CTT ATT CGT     1015
Asp Thr Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg
            310                 315                 320

AAT CCA AAA ATA CTA GCC CAA GCC CAG CAA GAG ATC GAC AAA GTC GTT     1063
Asn Pro Lys Ile Leu Ala Gln Ala Gln Gln Glu Ile Asp Lys Val Val
        325                 330                 335

GGA AGG GAC CGG CTA GTT GGC GAA TTG GAC CTA GCC CAA TTG ACA TAC     1111
Gly Arg Asp Arg Leu Val Gly Glu Leu Asp Leu Ala Gln Leu Thr Tyr
    340                 345                 350

TTG GAA GCT ATA GTC AAG GAA ACC TTT CGG CTT CAT CCA TCA ACC CCT     1159
Leu Glu Ala Ile Val Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro
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355 360 365 370

CTT TCA CTT CCT AGA ATT GCA TCT GAG AGT TGT GAG ATC AAT GGC TAT 1207
Leu Ser Leu Pro Arg Ile Ala Ser Glu Ser Cys Glu Ile Asn Gly Tyr
375 380 385

TTC ATT CCA AAA GGC TCA ACG CTT CTC CTT AAT GTT TGG GCC ATT GCT 1255
Phe Ile Pro Lys Gly Ser Thr Leu Leu Leu Asn Val Trp Ala Ile Ala
390 395 400

CGT GAT CCA AAT GCA TGG GCT GAT CCA TTG GAG TTT AGG CCT GAA AGG 1303
Arg Asp Pro Asn Ala Trp Ala Asp Pro Leu Glu Phe Arg Pro Glu Arg
405 410 415

TTT TTG CCA GGA GGT GAG AAG CCC AAA GTT GAT GTC CGT GGG AAT GAC 1351
Phe Leu Pro Gly Gly Glu Lys Pro Lys Val Asp Val Arg Gly Asn Asp
420 425 430

TTT GAA GTC ATA CCA TTT GGA GCT GGA CGT AGG ATT TGT GCT GGA ATG 1399
Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met
435 440 445 450

AAT TTG GGT ATA CGT ATG GTC CAG TTG ATG ATT GCA ACT TTA ATA CAT 1447
Asn Leu Gly Ile Arg Met Val Gln Leu Met Ile Ala Thr Leu Ile His
455 460 465

GCA TTT AAC TGG GAT TTG GTC AGT GGA CAA TTG CCG GAG ATG TTG AAT 1495
Ala Phe Asn Trp Asp Leu Val Ser Gly Gln Leu Pro Glu Met Leu Asn
470 475 480

ATG GAA GAA GCA TAT GGG CTG ACC TTA CAA CGG GCT GAT CCA TTG GTT 1543
Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Asp Pro Leu Val
485 490 495

GTG CAC CCA AGG CCT CGC TTA GAA GCC CAA GCG TAC ATT GGG T 1586
Val His Pro Arg Pro Arg Leu Glu Ala Gln Ala Tyr Ile Gly
500 505 510

GAGCAGCAAC AGCCCATGGA GATAACATGA GTGTTAAATG TATGAGTCTC CATATCTTGT 1646

TTAGTTTGTT TATGCTTTGG ATTTAGTAGT TTTTATATTG ATAGATCAAT GTTTGCATTG 1706

TCAGTAAGAA TATCCGTTGC TTGTTTCATT AACTCCAGGT GGACAATAAA AGAAGTAATT 1766

TGTATGAAAA AAAAAAAAAA AAA 1789

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 512 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Leu Gln Phe Ile Leu Arg Ser Phe Phe Arg Lys Arg Tyr Pro Leu Pro
20 25 30

Leu Pro Pro Gly Pro Lys Pro Trp Pro Ile Ile Gly Asn Leu Val His
35 40 45

Leu Gly Pro Lys Pro His Gln Ser Thr Ala Ala Met Ala Gln Thr Tyr
50 55 60

Gly Pro Leu Met Tyr Leu Lys Met Gly Phe Val Asp Val Val Val Ala
65 70 75 80

Ala Ser Ala Ser Val Ala Ala Gln Phe Leu Lys Thr His Asp Ala Asn
85 90 95

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Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Glu His Met Ala Tyr Asn
                100                 105                 110

Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu
        115                 120                 125

Arg Lys Ile Cys Ser Val His Leu Phe Ser Thr Lys Ala Leu Asp Asp
    130                 135                 140

Phe Arg His Val Arg Gln Asp Glu Val Lys Thr Leu Thr Arg Ala Leu
145                 150                 155                 160

Ala Ser Ala Gly Gln Lys Pro Val Lys Leu Gly Gln Leu Leu Asn Val
                165                 170                 175

Cys Thr Thr Asn Ala Leu Ala Arg Val Met Leu Gly Lys Arg Val Phe
            180                 185                 190

Ala Asp Gly Ser Gly Asp Val Asp Pro Gln Ala Ala Glu Phe Lys Ser
        195                 200                 205

Met Val Val Glu Met Met Val Val Ala Gly Val Phe Asn Ile Gly Asp
    210                 215                 220

Phe Ile Pro Gln Leu Asn Trp Leu Asp Ile Gln Gly Val Ala Ala Lys
225                 230                 235                 240

Met Lys Lys Leu His Ala Arg Phe Asp Ala Phe Leu Thr Asp Ile Leu
                245                 250                 255

Glu Glu His Lys Gly Lys Ile Phe Gly Glu Met Lys Asp Leu Leu Ser
            260                 265                 270

Thr Leu Ile Ser Leu Lys Asn Asp Asp Ala Asp Asn Asp Gly Gly Lys
        275                 280                 285

Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Val Ala
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290 295 300

Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu
305 310 315 320

Ile Arg Asn Pro Lys Ile Leu Ala Gln Ala Gln Gln Glu Ile Asp Lys
325 330 335

Val Val Gly Arg Asp Arg Leu Val Gly Glu Leu Asp Leu Ala Gln Leu
340 345 350

Thr Tyr Leu Glu Ala Ile Val Lys Glu Thr Phe Arg Leu His Pro Ser
355 360 365

Thr Pro Leu Ser Leu Pro Arg Ile Ala Ser Glu Ser Cys Glu Ile Asn
370 375 380

Gly Tyr Phe Ile Pro Lys Gly Ser Thr Leu Leu Leu Asn Val Trp Ala
385 390 395 400

Ile Ala Arg Asp Pro Asn Ala Trp Ala Asp Pro Leu Glu Phe Arg Pro
405 410 415

Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Lys Val Asp Val Arg Gly
420 425 430

Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala
435 440 445

Gly Met Asn Leu Gly Ile Arg Met Val Gln Leu Met Ile Ala Thr Leu
450 455 460

Ile His Ala Phe Asn Trp Asp Leu Val Ser Gly Gln Leu Pro Glu Met
465 470 475 480

Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Asp Pro
485 490 495

Leu Val Val His Pro Arg Pro Arg L u Glu Ala Gln Ala Tyr Ile Gly
500 505 510

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1745 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 172..1660

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGTTCGGCA CGAGCGTCAC ATTCACACCG TCACATTACT ATTCAAACCA CTCATTTTCT 60

ACCTCTCTTT TCTACCCACC AAAACAAAAC AAAACAAAAA AAAACACATA AAAAAACTCA 120

AAAAAAAATT ATAATGTCAC CCTTAGAGGT AACTTTCTAC ACCATAGTCC T ATG CAC 177
Met His
1

AAT CTC TAC TAC CTC ATC ACC ACC GTC TTC CGC GGC CAC CAA AAA CCG 225
Asn Leu Tyr Tyr Leu Ile Thr Thr Val Phe Arg Gly His Gln Lys Pro
5 10 15

CTT CCT CCA GGG CCA CGA CCA TGG CCC ATC GTG GGA AAC CTC CCA CAT 273
Leu Pro Pro Gly Pro Arg Pro Trp Pro Ile Val Gly Asn Leu Pro His
20 25 30

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ATG GGC CAG GCA CCG CAC CAG GGC TTA GCA GCC CTG GCG CAA AAG TAT      321
Met Gly Gln Ala Pro His Gln Gly Leu Ala Ala Leu Ala Gln Lys Tyr
 35                  40                  45                  50

GGC CCT CTA TTG TAT ATG AGA CTG GGG TAC GTG GAC GTT GTT GTG GCC      369
Gly Pro Leu Leu Tyr Met Arg Leu Gly Tyr Val Asp Val Val Val Ala
                 55                  60                  65

GCC TCA GCG TCT GTA GCG ACC CAG TTT CTT AAG ACA CAT GAC CTA AAT      417
Ala Ser Ala Ser Val Ala Thr Gln Phe Leu Lys Thr His Asp Leu Asn
             70                  75                  80

TTT TCG AGT AGG CCA CCG AAT TCG GGG GCT AAA CAC ATT GCT TAT AAC      465
Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His Ile Ala Tyr Asn
         85                  90                  95

TAT CAA GAC CTT GTT TTT GCA CCT TAT GGA CCT AAA TGG CGC ATG CTT      513
Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Lys Trp Arg Met Leu
    100                 105                 110

AGG AAA ATT TGT TCC TTA CAC ATG TTT TCT TCT AAG GCT TTG GAC GAT      561
Arg Lys Ile Cys Ser Leu His Met Phe Ser Ser Lys Ala Leu Asp Asp
115                 120                 125                 130

TTT AGA CTT GTC CGT CAG GAA GAA GTA TCT ATA CTG GTA AAT GCG ATA      609
Phe Arg Leu Val Arg Gln Glu Glu Val Ser Ile Leu Val Asn Ala Ile
                135                 140                 145

GCA AAA GCA GGA ACA AAG CCA GTA CAA CTA GGA CAA CTA CTC AAC GTG      657
Ala Lys Ala Gly Thr Lys Pro Val Gln Leu Gly Gln Leu Leu Asn Val
            150                 155                 160

TGC ACC ACA AAT GCC TTA TCG AGG GTG ATG CTA GGG AAG CGA GTT CTC      705
Cys Thr Thr Asn Ala Leu Ser Arg Val Met Leu Gly Lys Arg Val Leu
        165                 170                 175

GGT GAT GGC ACA GGG AAA AGC GAC CCA AAA GCC GAG GAA TTT AAG GAC      753
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Gly Asp Gly Thr Gly Lys Ser Asp Pro Lys Ala Glu Glu Phe Lys Asp
    180                 185                 190

ATG GTG CTG GAG TTA ATG GTT CTC ACC GGA GTT TTT AAC ATT GGC GAT      801
Met Val Leu Glu Leu Met Val Leu Thr Gly Val Phe Asn Ile Gly Asp
195                 200                 205                 210

TTT GTA CCG GCA TTG GAA TGT CTA GAC TTA CAA GGT GTT GCA TCT AAA      849
Phe Val Pro Ala Leu Glu Cys Leu Asp Leu Gln Gly Val Ala Ser Lys
                215                 220                 225

ATG AAG AAA TTA CAT AAA AGA CTT GAT AAT TTT ATG AGT AAC ATT TTG      897
Met Lys Lys Leu His Lys Arg Leu Asp Asn Phe Met Ser Asn Ile Leu
            230                 235                 240

GAG GAA CAC AAG AGT GTT GCA CAT CAA CAA AAT GGT GGA GAT TTG CTA      945
Glu Glu His Lys Ser Val Ala His Gln Gln Asn Gly Gly Asp Leu Leu
        245                 250                 255

AGC ATT TTG ATA TCT TTG AAG GAT AAT TGT GAT GGT GAA GGT GGC AAG      993
Ser Ile Leu Ile Ser Leu Lys Asp Asn Cys Asp Gly Glu Gly Gly Lys
    260                 265                 270

TTT AGT GCC ACA GAA ATT AAG GCC TTG CTA TTG GAT TTA TTT ACA GCT     1041
Phe Ser Ala Thr Glu Ile Lys Ala Leu Leu Leu Asp Leu Phe Thr Ala
275                 280                 285                 290

GGA ACA GAC ACA TCA TCT AGT ACA ACT GAA TGG GCC ATA GCC GAA CTA     1089
Gly Thr Asp Thr Ser Ser Ser Thr Thr Glu Trp Ala Ile Ala Glu Leu
                295                 300                 305

ATT CGC CAT CCA AAA ATC TTA GCC CAA GTT CAA CAA GAA ATG GAC TCA     1137
Ile Arg His Pro Lys Ile Leu Ala Gln Val Gln Gln Glu Met Asp Ser
            310                 315                 320

GTC GTG GGC CGA GAC CGA CTC ATA GCC GAA GCT GAC ATA CCG AAC CTA     1185
Val Val Gly Arg Asp Arg Leu Ile Ala Glu Ala Asp Ile Pro Asn Leu
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          325                 330                 335

ACC TAC TTC CAA GCC GTA ATC AAA GAG GTT TTC CGA CTT CAC CCG TCC      1233
Thr Tyr Phe Gln Ala Val Ile Lys Glu Val Phe Arg Leu His Pro Ser
    340                 345                 350

ACC CCG CTT TCC CTA CCA CGG GTC GCA AAC GAA TCG TGC GAA ATA AAC      1281
Thr Pro Leu Ser Leu Pro Arg Val Ala Asn Glu Ser Cys Glu Ile Asn
355                 360                 365                 370

GGG TAC CAC ATT CCC AAA AAC ACC ACT TTA TTG GTA AAT GTG TGG GCC      1329
Gly Tyr His Ile Pro Lys Asn Thr Thr Leu Leu Val Asn Val Trp Ala
                375                 380                 385

ATC GCA CGC GAC CCT GAG GTT TGG GCC GAC CCG TTA GAG TTT AAA CCC      1377
Ile Ala Arg Asp Pro Glu Val Trp Ala Asp Pro Leu Glu Phe Lys Pro
            390                 395                 400

GAA AGA TTT TTG CCG GGC GGC GAA AAG CCC AAT GTG GAT GTG AAA GGA      1425
Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val Asp Val Lys Gly
        405                 410                 415

AAC GAT TTT GAG CTG ATT CCG TTC GGG GCG GGC CGA CGG ATT TGT GCT      1473
Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala
    420                 425                 430

GGG CTG AGT TTG GGC CTG CGT ATG GTC CAG TTG ATG ACA GCC ACT TTG      1521
Gly Leu Ser Leu Gly Leu Arg Met Val Gln Leu Met Thr Ala Thr Leu
435                 440                 445                 450

GCC CAT ACT TAT GAT TGG GCC TTA GCT GAT GGG CTT ATG CCC GAA AAG      1569
Ala His Thr Tyr Asp Trp Ala Leu Ala Asp Gly Leu Met Pro Glu Lys
                455                 460                 465

CTT AAC ATG GAT GAG GCT TAT GGG CTT ACC TTA CAG CGT AAG GTG CCA      1617
Leu Asn Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln Arg Lys Val Pro
            470                 475                 480
```

CTT AAT GGT CCA CCC GAC CCC GTC GGC TTC TCG GCC CGT GTT T 1660

Leu Asn Gly Pro Pro Asp Pro Val Gly Phe Ser Ala Arg Val
485 490 495

AATAATTCCG GGGTTTTTAA AAGCGGGTTA CTTTTGTTTA TGTATTATTC CGTACTAGTT 1720

TGAAAATAAT GGTATTAGAG AAATG 1745

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 496 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met His Asn Leu Tyr Tyr Leu Ile Thr Thr Val Phe Arg Gly His Gln
1 5 10 15

Lys Pro Leu Pro Pro Gly Pro Arg Pro Trp Pro Ile Val Gly Asn Leu
20 25 30

Pro His Met Gly Gln Ala Pro His Gln Gly Leu Ala Ala Leu Ala Gln
35 40 45

Lys Tyr Gly Pro Leu Leu Tyr Met Arg Leu Gly Tyr Val Asp Val Val
50 55 60

Val Ala Ala Ser Ala Ser Val Ala Thr Gln Phe Leu Lys Thr His Asp
65 70 75 80

Leu Asn Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His Ile Ala
85 90 95

Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Lys Trp Arg
100 105 110

Met Leu Arg Lys Ile Cys Ser Leu His Met Phe Ser Ser Lys Ala Leu
115 120 125

Asp Asp Phe Arg Leu Val Arg Gln Glu Glu Val Ser Ile Leu Val Asn
130 135 140

Ala Ile Ala Lys Ala Gly Thr Lys Pro Val Gln Leu Gly Gln Leu Leu
145 150 155 160

Asn Val Cys Thr Thr Asn Ala Leu Ser Arg Val Met Leu Gly Lys Arg
165 170 175

Val Leu Gly Asp Gly Thr Gly Lys Ser Asp Pro Lys Ala Glu Glu Phe
180 185 190

Lys Asp Met Val Leu Glu Leu Met Val Leu Thr Gly Val Phe Asn Ile
195 200 205

Gly Asp Phe Val Pro Ala Leu Glu Cys Leu Asp Leu Gln Gly Val Ala
210 215 220

Ser Lys Met Lys Lys Leu His Lys Arg Leu Asp Asn Phe Met Ser Asn
225 230 235 240

Ile Leu Glu Glu His Lys Ser Val Ala His Gln Gln Asn Gly Gly Asp
245 250 255

Leu Leu Ser Ile Leu Ile Ser Leu Lys Asp Asn Cys Asp Gly Glu Gly
260 265 270

Gly Lys Phe Ser Ala Thr Glu Ile Lys Ala Leu Leu Leu Asp Leu Phe
275 280 285

Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Thr Glu Trp Ala Ile Ala

290 295 300

Glu Leu Ile Arg His Pro Lys Ile Leu Ala Gln Val Gln Gln Glu Met
305 310 315 320

Asp Ser Val Val Gly Arg Asp Arg Leu Ile Ala Glu Ala Asp Ile Pro
325 330 335

Asn Leu Thr Tyr Phe Gln Ala Val Ile Lys Glu Val Phe Arg Leu His
340 345 350

Pro Ser Thr Pro Leu Ser Leu Pro Arg Val Ala Asn Glu Ser Cys Glu
355 360 365

Ile Asn Gly Tyr His Ile Pro Lys Asn Thr Thr Leu Leu Val Asn Val
370 375 380

Trp Ala Ile Ala Arg Asp Pro Glu Val Trp Ala Asp Pro Leu Glu Phe
385 390 395 400

Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val Asp Val
405 410 415

Lys Gly Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile
420 425 430

Cys Ala Gly Leu Ser Leu Gly Leu Arg Met Val Gln Leu Met Thr Ala
435 440 445

Thr Leu Ala His Thr Tyr Asp Trp Ala Leu Ala Asp Gly Leu Met Pro
450 455 460

Glu Lys Leu Asn Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln Arg Lys
465 470 475 480

Val Pro Leu Asn Gly Pro Pro Asp Pro Val Gly Phe Ser Ala Arg Val
485 490 495

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1711 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 91..1629

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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CGAATTCCCC CCCCCCCACA CCATTCAATG CCTAAGTCCT CCATTTGCCG GCCTAATAAC      60

TAAAAGCCCA CTCTTTCCGA CCATCTATAC ATG CAA CAC CAA TAT TAT TCT TTA      114
                                 Met Gln His Gln Tyr Tyr Ser Leu
                                   1               5

ATT ACG ATG GAT GAT ATT AGC ATA ACC AGC TTA TTG GTG CCA TGT ACT      162
Ile Thr Met Asp Asp Ile Ser Ile Thr Ser Leu Leu Val Pro Cys Thr
     10                  15                  20

TTT ATA TTA GGG TTC TTG CTT CTA TAT TCC TTC CTC AAC AAA AAA GTA      210
Phe Ile Leu Gly Phe Leu Leu Leu Tyr Ser Phe Leu Asn Lys Lys Val
 25                  30                  35                  40

AAG CCA CTG CCA CCT GGA CCG AAG CCA TGG CCC ATC GTC GGA AAT CTG      258
Lys Pro Leu Pro Pro Gly Pro Lys Pro Trp Pro Ile Val Gly Asn Leu
                 45                  50                  55

CCA CAT CTT GGG CCG AAG CCC CAC CAG TCG ATG GCG GCG CTG GCA CGG      306
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Pro His Leu Gly Pro Lys Pro His Gln Ser Met Ala Ala Leu Ala Arg
                60                  65                  70

GTG CAC GGC CCA TTA ATT CAT CTG AAG ATG GGC TTT GTG CAT GTG GTT      354
Val His Gly Pro Leu Ile His Leu Lys Met Gly Phe Val His Val Val
         75                  80                  85

GTG GCC TCC TCA GCA TCC GTT GCG GAG AAA TTT CTG AAG GTG CAT GAC      402
Val Ala Ser Ser Ala Ser Val Ala Glu Lys Phe Leu Lys Val His Asp
     90                  95                 100

GCA AAC TTC TCG AGC AGG CCT CCC AAT TCG GGT GCA AAA CAC GTG GCC      450
Ala Asn Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala
105                 110                 115                 120

TAC AAC TAT CAG GAC TTG GTC TTT GCT CCT TAT GGC CCA CGC TGG CGG      498
Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg
                125                 130                 135

ATG CTC AGG AAA ATC TGT GCA CTC CAC CTC TTC TCC GCC AAA GCC TTG      546
Met Leu Arg Lys Ile Cys Ala Leu His Leu Phe Ser Ala Lys Ala Leu
            140                 145                 150

AAC GAC TTC ACA CAC GTC AGA CAG GAT GAG GTG GGG ATC CTC ACT CGC      594
Asn Asp Phe Thr His Val Arg Gln Asp Glu Val Gly Ile Leu Thr Arg
        155                 160                 165

GTT CTA GCA GAT GCA GGA GAA ACG CCG TTG AAA TTA GGG CAG ATG ATG      642
Val Leu Ala Asp Ala Gly Glu Thr Pro Leu Lys Leu Gly Gln Met Met
    170                 175                 180

AAC ACA TGC GCC ACC AAT GCA ATA GCG CGT GTT ATG TTG GGT CGA CGC      690
Asn Thr Cys Ala Thr Asn Ala Ile Ala Arg Val Met Leu Gly Arg Arg
185                 190                 195                 200

GTG GTT GGA CAC GCA GAC TCA AAG GCG GAG GAG TTT AAG GCA ATG GTA      738
Val Val Gly His Ala Asp Ser Lys Ala Glu Glu Phe Lys Ala Met Val
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205 210 215

GTG GAG TTG ATG GTA TTA GCT GGT GTG TTC AAC TTA GGT GAT TTT ATC 786
Val Glu Leu Met Val Leu Ala Gly Val Phe Asn Leu Gly Asp Phe Ile
220 225 230

CCA CCT CTT GAA AAA TTG GAT CTT CAA GGT GTC ATT GCT AAG ATG AAG 834
Pro Pro Leu Glu Lys Leu Asp Leu Gln Gly Val Ile Ala Lys Met Lys
235 240 245

AAG CTT CAC TTG CGT TTC GAC TCG TTC TTG AGT AAG ATC CTT GGA GAC 882
Lys Leu His Leu Arg Phe Asp Ser Phe Leu Ser Lys Ile Leu Gly Asp
250 255 260

CAC AAG ATC AAC AGC TCA GAT GAA ACC AAA GGC CAT TCG GAT TTG TTG 930
His Lys Ile Asn Ser Ser Asp Glu Thr Lys Gly His Ser Asp Leu Leu
265 270 275 280

AAC ATG TTA ATT TCT TTG AAG GAC GCT GAT GAT GCC GAA GGA GGG AGG 978
Asn Met Leu Ile Ser Leu Lys Asp Ala Asp Asp Ala Glu Gly Gly Arg
285 290 295

CTC ACC GAC GTA GAA ATT AAA GCG TTG CTC TTG AAC TTG TTT GCT GCA 1026
Leu Thr Asp Val Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ala Ala
300 305 310

GGA ACT GAC ACA ACA TCA AGC ACT GTG GAA TGG TGC ATA GCT GAG TTA 1074
Gly Thr Asp Thr Thr Ser Ser Thr Val Glu Trp Cys Ile Ala Glu Leu
315 320 325

GTA CGA CAT CCT GAA ATC CTT GCC CAA GTC CAA AAA GAA CTC GAC TCT 1122
Val Arg His Pro Glu Ile Leu Ala Gln Val Gln Lys Glu Leu Asp Ser
330 335 340

GTT GTT GGT AAG AAT CGG GTG GTG AAG GAG GCT GAT CTG GCC GGA TTA 1170
Val Val Gly Lys Asn Arg Val Val Lys Glu Ala Asp Leu Ala Gly Leu
345 350 355 360

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CCA TTC CTC CAA GCG GTC GTC AAG GAA AAT TTC CGA CTC CAT CCC TCC      1218
Pro Phe Leu Gln Ala Val Val Lys Glu Asn Phe Arg Leu His Pro Ser
                365                 370                 375

ACC CCG CTC TCC CTA CCG AGG ATC GCA CAT GAG AGT TGT GAA GTG AAT      1266
Thr Pro Leu Ser Leu Pro Arg Ile Ala His Glu Ser Cys Glu Val Asn
            380                 385                 390

GGA TAC TTG ATT CCA AAG GGT TCG ACA CTT CTT GTC AAT GTT TGG GCA      1314
Gly Tyr Leu Ile Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala
        395                 400                 405

ATT GCT CGC GAT CCA AAT GTG TGG GAT GAA CCA CTA GAG TTC CGG CCT      1362
Ile Ala Arg Asp Pro Asn Val Trp Asp Glu Pro Leu Glu Phe Arg Pro
    410                 415                 420

GAA CGA TTC TTG AAG GGC GGG GAA AAG CCT AAT GTC GAT GTT AGA GGG      1410
Glu Arg Phe Leu Lys Gly Gly Glu Lys Pro Asn Val Asp Val Arg Gly
425                 430                 435                 440

AAT GAT TTC GAA TTG ATA CCG TTC GGA GCG GGC CGA AGA ATT TGT GCA      1458
Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala
                445                 450                 455

GGA ATG AGC TTA GGA ATA CGT ATG GTC CAG TTG TTG ACA GCA ACT TTG      1506
Gly Met Ser Leu Gly Ile Arg Met Val Gln Leu Leu Thr Ala Thr Leu
            460                 465                 470

AAC CAT GCG TTT GAC TTT GAT TTG GCG GAT GGA CAG TTG CCT GAA AGC      1554
Asn His Ala Phe Asp Phe Asp Leu Ala Asp Gly Gln Leu Pro Glu Ser
        475                 480                 485

TTA AAC ATG GAG GAA GCT TAT GGG CTG ACC TTG CAA CGA GCT GAC CCT      1602
Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Asp Pro
    490                 495                 500

TTG GTA GTG CAC CCG AAG CCT AGG TAGGCACCTC ATGTTTATCA AACTTAGGAC      1656
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Leu Val Val His Pro Lys Pro Arg
505 510

TCATGTTTAG AGAACCTCTT GTTGTTTTAT CAGATTGAAG TGTGATGTCC AAGAC 1711

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 512 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gln His Gln Tyr Tyr Ser Leu Ile Thr Met Asp Asp Ile Ser Ile
1 5 10 15

Thr Ser Leu Leu Val Pro Cys Thr Phe Ile Leu Gly Phe Leu Leu Leu
20 25 30

Tyr Ser Phe Leu Asn Lys Lys Val Lys Pro Leu Pro Pro Gly Pro Lys
35 40 45

Pro Trp Pro Ile Val Gly Asn Leu Pro His Leu Gly Pro Lys Pro His
50 55 60

Gln Ser Met Ala Ala Leu Ala Arg Val His Gly Pro Leu Ile His Leu
65 70 75 80

Lys Met Gly Phe Val His Val Val Val Ala Ser Ser Ala Ser Val Ala
85 90 95

Glu Lys Phe Leu Lys Val His Asp Ala Asn Phe Ser Ser Arg Pro Pro
100 105 110

Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr Gln Asp Leu Val Phe
115 120 125

Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Ile Cys Ala Leu
130 135 140

His Leu Phe Ser Ala Lys Ala Leu Asn Asp Phe Thr His Val Arg Gln
145 150 155 160

Asp Glu Val Gly Ile Leu Thr Arg Val Leu Ala Asp Ala Gly Glu Thr
165 170 175

Pro Leu Lys Leu Gly Gln Met Met Asn Thr Cys Ala Thr Asn Ala Ile
180 185 190

Ala Arg Val Met Leu Gly Arg Arg Val Val Gly His Ala Asp Ser Lys
195 200 205

Ala Glu Glu Phe Lys Ala Met Val Val Glu Leu Met Val Leu Ala Gly
210 215 220

Val Phe Asn Leu Gly Asp Phe Ile Pro Pro Leu Glu Lys Leu Asp Leu
225 230 235 240

Gln Gly Val Ile Ala Lys Met Lys Lys Leu His Leu Arg Phe Asp Ser
245 250 255

Phe Leu Ser Lys Ile Leu Gly Asp His Lys Ile Asn Ser Ser Asp Glu
260 265 270

Thr Lys Gly His Ser Asp Leu Leu Asn Met Leu Ile Ser Leu Lys Asp
275 280 285

Ala Asp Asp Ala Glu Gly Gly Arg Leu Thr Asp Val Glu Ile Lys Ala
290 295 300

Leu Leu Leu Asn Leu Phe Ala Ala Gly Thr Asp Thr Thr Ser Ser Thr

305 310 315 320

Val Glu Trp Cys Ile Ala Glu Leu Val Arg His Pro Glu Ile Leu Ala
325 330 335

Gln Val Gln Lys Glu Leu Asp Ser Val Val Gly Lys Asn Arg Val Val
340 345 350

Lys Glu Ala Asp Leu Ala Gly Leu Pro Phe Leu Gln Ala Val Val Lys
355 360 365

Glu Asn Phe Arg Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Ile
370 375 380

Ala His Glu Ser Cys Glu Val Asn Gly Tyr Leu Ile Pro Lys Gly Ser
385 390 395 400

Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp Pro Asn Val Trp
405 410 415

Asp Glu Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu Lys Gly Gly Glu
420 425 430

Lys Pro Asn Val Asp Val Arg Gly Asn Asp Phe Glu Leu Ile Pro Phe
435 440 445

Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu Gly Ile Arg Met
450 455 460

Val Gln Leu Leu Thr Ala Thr Leu Asn His Ala Phe Asp Phe Asp Leu
465 470 475 480

Ala Asp Gly Gln Leu Pro Glu Ser Leu Asn Met Glu Glu Ala Tyr Gly
485 490 495

Leu Thr Leu Gln Arg Ala Asp Pro Leu Val Val His Pro Lys Pro Arg
500 505 510

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 971 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..811

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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GAT ATG CTT AGC ACT TTA ATC TCC CTT AAA GGA ACT GAT CTT GAC GGT        48
Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly Thr Asp Leu Asp Gly
  1               5                  10                  15

GAC GGA GGA AGC TTA ACG GAT ACT GAG ATT AAA GCC TTG CTA TTG AAC        96
Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn
            20                  25                  30

ATG TTC ACA GCT GGA ACT GAC ACG TCA GCA AGT ACG GTG GAC TGG GCT       144
Met Phe Thr Ala Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp Ala
         35                  40                  45

ATA GCT GAA CTT ATC CGT CAC CCG GAT ATA ATG GTT AAA GCC CAA GAA       192
Ile Ala Glu Leu Ile Arg His Pro Asp Ile Met Val Lys Ala Gln Glu
     50                  55                  60

GAA CTT GAT ATT GTT GTG GGC CGT GAC AGG CCT GTT AAT GAA TCA GAC       240
Glu Leu Asp Ile Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser Asp
 65                  70                  75                  80
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ATC GCT CAG CTT CCT TAC CTT CAG GCG GTT ATC AAA GAG AAT TTC AGG        288
Ile Ala Gln Leu Pro Tyr Leu Gln Ala Val Ile Lys Glu Asn Phe Arg
                85                  90                  95

CTT CAT CCA CCA ACA CCA CTC TCG TTA CCA CAC ATC GCG TCA GAG AGC        336
Leu His Pro Pro Thr Pro Leu Ser Leu Pro His Ile Ala Ser Glu Ser
            100                 105                 110

TGT GAG ATC AAC GGC TAC CAT ATC CCG AAA GGA TCG ACT CTA TTT GAC        384
Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr Leu Phe Asp
        115                 120                 125

GGA CAT ATG GGC CTA GGC CGT GAC CCG GAT CAA TGG TCC GAC CCG TTA        432
Gly His Met Gly Leu Gly Arg Asp Pro Asp Gln Trp Ser Asp Pro Leu
    130                 135                 140

GCA TTT AAA CCC GAG AGA TTC TTA CCC GGT GGT GAA AAA TCC GGC GTT        480
Ala Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Ser Gly Val
145                 150                 155                 160

GAT GTG AAA GGA AGC GAT TTC GAG CTA ATA CCG TTC GGG GCT GGG AGG        528
Asp Val Lys Gly Ser Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg
                165                 170                 175

CCA ATC TGT GCA GGT TTA AGT TTA GGG CTA CGT ACA GAT TTA AGT TGC        576
Pro Ile Cys Ala Gly Leu Ser Leu Gly Leu Arg Thr Asp Leu Ser Cys
            180                 185                 190

CTT CAC GCC AAC GTT GCT CAC AAG CAT TTG ATT GGG AAC TTC AGC TGG        624
Leu His Ala Asn Val Ala His Lys His Leu Ile Gly Asn Phe Ser Trp
        195                 200                 205

AGA AGT TAC GCC GGA CAA CCT GAA TAT CGC AGG AAA AGT TTA CTG GGC        672
Arg Ser Tyr Ala Gly Gln Pro Glu Tyr Arg Arg Lys Ser Leu Leu Gly
    210                 215                 220

TTT ACA CTG CAA AGA GCG GTT CCT TCG GTG GTA CAC CCT AAG CCA AGG        720
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Phe Thr Leu Gln Arg Ala Val Pro Ser Val Val His Pro Lys Pro Arg
225 230 235 240

TTG GCC CCG AAC GTT TAT GGA CCC CGG GTC GGC TTA AAA TTT AAC TTT 768
Leu Ala Pro Asn Val Tyr Gly Pro Arg Val Gly Leu Lys Phe Asn Phe
245 250 255

GCT TCT TGG ACA AGG TAT ATG GCT TGC ACG AAA CTA ACG TTT T 811
Ala Ser Trp Thr Arg Tyr Met Ala Cys Thr Lys Leu Thr Phe
260 265 270

AACACACCGT AGTTTGATCC GGAGTTAGCT TTATGTAAGA ACGTGTAACG CCAAATCAAG 871

CCATTATCAA CTACCGTGAG CTGTTTGTAC CCTATCTATA AATCTTGAAG AGGAACATTT 931

CAGAACTCTT GACTATGTTT CAGGAACAAA AAAAAAAAAA 971

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 270 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly Thr Asp Leu Asp Gly
1 5 10 15

Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn
20 25 30

Met Phe Thr Ala Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp Ala
35 40 45

Ile Ala Glu Leu Ile Arg His Pro Asp Ile Met Val Lys Ala Gln Glu
50 55 60

Glu Leu Asp Ile Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser Asp
65 70 75 80

Ile Ala Gln Leu Pro Tyr Leu Gln Ala Val Ile Lys Glu Asn Phe Arg
85 90 95

Leu His Pro Pro Thr Pro Leu Ser Leu Pro His Ile Ala Ser Glu Ser
100 105 110

Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr Leu Phe Asp
115 120 125

Gly His Met Gly Leu Gly Arg Asp Pro Asp Gln Trp Ser Asp Pro Leu
130 135 140

Ala Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Ser Gly Val
145 150 155 160

Asp Val Lys Gly Ser Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg
165 170 175

Pro Ile Cys Ala Gly Leu Ser Leu Gly Leu Arg Thr Asp Leu Ser Cys
180 185 190

Leu His Ala Asn Val Ala His Lys His Leu Ile Gly Asn Phe Ser Trp
195 200 205

Arg Ser Tyr Ala Gly Gln Pro Glu Tyr Arg Arg Lys Ser Leu Leu Gly
210 215 220

Phe Thr Leu Gln Arg Ala Val Pro Ser Val Val His Pro Lys Pro Arg
225 230 235 240

Leu Ala Pro Asn Val Tyr Gly Pro Arg Val Gly Leu Lys Phe Asn Phe

245 250 255

Ala Ser Trp Thr Arg Tyr Met Ala Cys Thr Lys Leu Thr Phe
260 265 270

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6595 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1478..1927

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2651..3091

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3170..3340

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3421..3900

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTCGACTCTC TCCCTTTCGC TTGCTACTTT TTCTACATAA ATAAATGCAA TGATAAATTT 60

GTGCACACAT TCGTATGTTT GAAACATGGT AGGATCCACA ATTTATACTT TATAGACTCA 120

AAATGGAAAA GAAACGTACA TTATAAATTT ATCTGCAATT TGTTTTCTCT TGCTAAACTA 180

GACTGTATAA TAACCTCTGT ATATGCTATT ACTCGATTGT AAACGTACCC CGCAAGTCGC 240

AAGCAAGGTA AATAAAGTAT AATTATATTT TCACACACGA AACTTTAATT ATTATTTTTA 300

TCACTTGCAG ATTAACAGTA AAAAAAAAAA AAATGTGACT TTAACGGCGA CAAAAACTAC 360

TGATCTTTCT CCAATATTTA AATAATATAA TTAATAAACG TCTTTTCATA CTTGTATTTT 420

CCGACCCGAG TTCTGAAAGT GAAAACATAT GGTACTAGAT ATTCTCGATT TGTTTTGTAG 480

CCACTAGACT CTAAACAGAA AAAAGAAGCC AAAAGGACAA CGTTAAAAAA GAGACACTGT 540

TATTAAAAGT TAGAAACCAA ACGGTGAAAA TCCAGCTACA TACATAAAAT AAAGCCAAGG 600

TACCAAACTA ATGAACTGTA ACCTCTTTTT TCTTTTCTTT TTTGTTAAAG GATTTATGAA 660

CTGTAACTTA GAATGCTTGG TTTGTGGGCA GTGTAATATA TGACACACAT GCATTTTTTT 720

TGTTTGTCAA ATAGGAAGAC TTCTTTTTTC TTTATCAACT TCCTTATTTT CATAAAACAA 780

AACACTGAAA AAAGTACAGA TGTTCTCACG TACGTCACGT GTACATACAT ATATATTAGA 840

CCACTATATA ATAAGATATG AAGTGTTAGG TTTAAATCAA TTAACGAATC CCATCCAAAT 900

GATGAAACAG TTAACAAGAA ATCAAAATAG TTTATTAGGG TTACAATGAT TTTATACTTT 960

TAAGAAATCT TAGAACCTAT CACTTACAAA TGAGTAAATG ACCATTACTC CTCGAGAATC 1020

TAAGGCGCTT AAGGAAGCAT TGCGAATCGG GTGTGAAAAA GATCTATTTT TTGAATTATT 1080

TCACACAATT TCTTAATGTC AATTTTCGAT GCTCCCATAT TCTCCACGGT TTAAAGCAAG 1140

ATTGGTGGGA AAGGGATATT CTCGCATCGA TTACAATGAA ATATGGGTTG AAAAAAAAAA 1200

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AAAAAAATTA CTCAATGTTG CACCAAAAAC CAGAAAACTC TAAGTTGCGC TAATAAAAAA    1260

AAAAGTTATA AACCCAACAT CAAACCAAAA CCGTACTAAA CTGTCCCATA TGAGATTTAG    1320

CTTTAAATAA ATTAGTACTT CTCATAACGA TAACTAAATT AAATTTCCCT AGCCAAGACA    1380

TACATATAGT TTTGATTGAC AAAAAAAAAA AAAACTCCTC TATTTATAGC TTGTGTTTTG    1440

TTTCCTCATT TTTCACTTAC CATTCAAACC CAACACT ATG GCA ACT CTA TTT CTC      1495
                                          Met Ala Thr Leu Phe Leu
                                           1               5

ACA ATC CTC CTA GCC ACT GTC CTC TTC CTC ATC CTC CGT ATC TTC TCT       1543
Thr Ile Leu Leu Ala Thr Val Leu Phe Leu Ile Leu Arg Ile Phe Ser
            10                  15                  20

CAC CGT CGC AAC CGC AGC CAC AAC AAC CGT CTT CCA CCG GGG CCA AAC       1591
His Arg Arg Asn Arg Ser His Asn Asn Arg Leu Pro Pro Gly Pro Asn
        25                  30                  35

CCA TGG CCC ATC ATC GGA AAC CTC CCT CAC ATG GGC ACT AAG CCT CAT      1639
Pro Trp Pro Ile Ile Gly Asn Leu Pro His Met Gly Thr Lys Pro His
    40                  45                  50

CGA ACC CTT TCC GCC ATG GTT ACT ACT TAC GGC CCT ATC CTC CAC CTC      1687
Arg Thr Leu Ser Ala Met Val Thr Thr Tyr Gly Pro Ile Leu His Leu
55                  60                  65                  70

CGA CTA GGG TTC GTA GAC GTC GTG GTC GCC GCT TCT AAA TCC GTG GCC      1735
Arg Leu Gly Phe Val Asp Val Val Val Ala Ala Ser Lys Ser Val Ala
                75                  80                  85

GAG CAG TTC TTG AAA ATA CAC GAC GCC AAT TTC GCT AGC CGA CCA CCA      1783
Glu Gln Phe Leu Lys Ile His Asp Ala Asn Phe Ala Ser Arg Pro Pro
            90                  95                  100

AAC TCA GGA GCC AAA CAC ATG GCA TAT AAC TAT CAA GAT CTT GTC TTT      1831
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Asn Ser Gly Ala Lys His Met Ala Tyr Asn Tyr Gln Asp Leu Val Phe
        105                 110                 115

GCA CCT TAC GGA CAC CGA TGG AGA CTG TTG AGA AAG ATT AGT TCT GTT      1879
Ala Pro Tyr Gly His Arg Trp Arg Leu Leu Arg Lys Ile Ser Ser Val
    120                 125                 130

CAT CTA TTT TCA GCT AAA GCT CTC GAA GAT TTC AAA CAT GTT CGA CAG      1927
His Leu Phe Ser Ala Lys Ala Leu Glu Asp Phe Lys His Val Arg Gln
135                 140                 145                 150

GTAAAACAAT TATAAACGGT ATTCTCATTT TCTAACGCTA TAGCTCACTG GCCTGTAATC    1987

ATGTCATTTC AATGTTTTGA CTTTTTCTTT ATATATACAT AATTATAATT TATAATTGGG    2047

ATTTCAAACC CTATCTCTCA CTATTTCAAG ACTAGACCGG ATTGGAATTT GAACTTTTGT    2107

AATGAATATT AGTATCTGCA CATAAATTTT ATGTTAAAGT TGGGTTTTCT TAAAGTGAAT    2167

TTATATATTA AAAATATATA AACGATTGGG TTTTACTCAA ATGAATTTAC ATAAGAGCTA    2227

GGTATAAGTG CAAATATGCA ATACTGTCAT TGTCGTGGAT GTATAAAAGT ATGATCTAAC    2287

TTTGATGATG CCATGGAAAA ATTGGAAAGT TCAGATCCAG AGGAAACATT GCTTGAATTA    2347

TAAAATGTAT GGACCACATT GTTTCCTTAA ATGGAAGGTC TCACGAGTTT CTCAATTTCA    2407

GACTACTGAT AATATATGCT ATTATAGATT TTATTTTCTG ATTATTTTTT TTGGTTTAAT    2467

TTAATTAGAG TAAATTTTTA AAAAGAAATA TATGGTTTTG TTAACCGTGT TTTAAAATTT    2527

GATAGAGCTT TTAGATCATA ATCATAATTT TTTCGTATTA ATTGTGATTA TGTTGGACGA    2587

AAATACTTAA TTAGTATTCA AGAAAACTCT TATTCTAAAA ACAGAAATAA ATGAATTTTA    2647

CAG GAA GAG GTT GGA ACG CTA ACG CGG GAG CTA GTG CGT GTT GGC ACG      2695
    Glu Glu Val Gly Thr Leu Thr Arg Glu Leu Val Arg Val Gly Thr
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          1               5                  10                  15

AAA CCC GTG AAT TTA GGC CAG TTG GTG AAC ATG TGT GTA GTC AAC GCT      2743
Lys Pro Val Asn Leu Gly Gln Leu Val Asn Met Cys Val Val Asn Ala
                20                  25                  30

CTA GGA CGA GAG ATG ATC GGA CGG CGA CTG TTC GGC GCC GAC GCC GAT      2791
Leu Gly Arg Glu Met Ile Gly Arg Arg Leu Phe Gly Ala Asp Ala Asp
            35                  40                  45

CAT AAA GCT GAC GAG TTT CGA TCG ATG GTG ACG GAA ATG ATG GCT CTC      2839
His Lys Ala Asp Glu Phe Arg Ser Met Val Thr Glu Met Met Ala Leu
        50                  55                  60

GCC GGA GTA TTT AAC ATC GGA GAT TTC GTG CCG TCA CTT GAT TGG TTA      2887
Ala Gly Val Phe Asn Ile Gly Asp Phe Val Pro Ser Leu Asp Trp Leu
    65                  70                  75

GAT TTA CAA GGC GTC GCT GGT AAA ATG AAA CGG CTT CAC AAA AGA TTC      2935
Asp Leu Gln Gly Val Ala Gly Lys Met Lys Arg Leu His Lys Arg Phe
80                  85                  90                  95

GAC GCT TTT CTA TCG TCG ATT TTG AAA GAG CAC GAA ATG AAC GGT CAA      2983
Asp Ala Phe Leu Ser Ser Ile Leu Lys Glu His Glu Met Asn Gly Gln
                100                 105                 110

GAT CAA AAG CAT ACA GAT ATG CTT AGC ACT TTA ATC TCC CTT AAA GGA      3031
Asp Gln Lys His Thr Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly
            115                 120                 125

ACT GAT CTT GAC GGT GAC GGA GGA AGC TTA ACG GAT ACT GAG ATT AAA      3079
Thr Asp Leu Asp Gly Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys
        130                 135                 140

GCC TTG CTA TTG GTCAGTTTTT TGACAATTAA TTTCCTTAAA AATCGTATAT          3131
Ala Leu Leu Leu
    145
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AATGAAAGTT AGATTGTTTT TTTTGGTTGT AAATACAG AAC ATG TTC ACA GCT        3184
                                                Asn Met Phe Thr Ala
                                                 1               5

GGA ACT GAC ACG TCA GCA AGT ACG GTG GAC TGG GCT ATA GCT GAA CTT      3232
Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp Ala Ile Ala Glu Leu
                 10                  15                  20

ATC CGT CAC CCG GAT ATA ATG GTT AAA GCC CAA GAA GAA CTT GAT ATT      3280
Ile Arg His Pro Asp Ile Met Val Lys Ala Gln Glu Glu Leu Asp Ile
             25                  30                  35

GTT GTG GGC CGT GAC AGG CCT GTT AAT GAA TCA GAC ATC GCT CAG CTT      3328
Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser Asp Ile Ala Gln Leu
         40                  45                  50

CCT TAC CTT CAG GTACCGTTAA CCCAAACCGG AATTTGGAAT TGTTTTGGTT          3380
Pro Tyr Leu Gln
     55

AGCGAGCTAT TGTTGTTAAT CCGGTTTTGG TTTAAAACAG GCG GTT ATC AAA GAG      3435
                                            Ala Val Ile Lys Glu
                                             1               5

AAT TTC AGG CTT CAT CCA CCA ACA CCA CTC TCG TTA CCA CAC ATC GCG      3483
Asn Phe Arg Leu His Pro Pro Thr Pro Leu Ser Leu Pro His Ile Ala
                 10                  15                  20

TCA GAG AGC TGT GAG ATC AAC GGC TAC CAT ATC CCG AAA GGA TCG ACT      3531
Ser Glu Ser Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr
             25                  30                  35

CTA TTG ACG AAC ATA TGG GCC ATA GCC CGT GAC CCG GAT CAA TGG TCC      3579
Leu Leu Thr Asn Ile Trp Ala Ile Ala Arg Asp Pro Asp Gln Trp Ser
         40                  45                  50

GAC CCG TTA GCA TTT AAA CCC GAG AGA TTC TTA CCC GGT GGT GAA AAA      3627
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Asp Pro Leu Ala Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys
     55                  60                  65

TCC GGC GTT GAT GTG AAA GGA AGC GAT TTC GAG CTA ATA CCG TTC GGA      3675
Ser Gly Val Asp Val Lys Gly Ser Asp Phe Glu Leu Ile Pro Phe Gly
 70                  75                  80                  85

GCT GGG AGG AGA ATC TGT GCC GGT TTA AGT TTA GGG TTA CGT ACG ATT      3723
Ala Gly Arg Arg Ile Cys Ala Gly Leu Ser Leu Gly Leu Arg Thr Ile
                 90                  95                 100

CAG TTT CTT ACG GCG ACG TTG GTT CAA GGA TTT GAT TGG GAA TTA GCT      3771
Gln Phe Leu Thr Ala Thr Leu Val Gln Gly Phe Asp Trp Glu Leu Ala
            105                 110                 115

GGA GGA GTT ACG CCG GAG AAG CTG AAT ATG GAG GAG AGT TAT GGG CTT      3819
Gly Gly Val Thr Pro Glu Lys Leu Asn Met Glu Glu Ser Tyr Gly Leu
        120                 125                 130

ACA CTG CAA AGA GCG GTT CCT TTG GTG GTA CAT CCT AAG CCA AGG TTG      3867
Thr Leu Gln Arg Ala Val Pro Leu Val Val His Pro Lys Pro Arg Leu
    135                 140                 145

GCT CCG AAC GTT TAT GGA CTC GGG TCG GGT TAAAATTTAA CTTTGCTTCT        3917
Ala Pro Asn Val Tyr Gly Leu Gly Ser Gly
150                 155                 160

TGGACAAGGT ATATGGCTTG CACGAAAATA AAGTTTTAAA ACAGCGTAGT TTGATCCGGA    3977

GTTAGCTTTA TGTAAGAACG TGTAACGCCA AATCAAGTCA TTATTAAATA TTGTGAGTTG    4037

TTTGTAACCT ATATATAAAT CTTGAAGAGG AAGATTTCAG AAATCTTGAA TATGTTTTAG    4097

GAAAAACATT GTTTTTTTTA CAGTAGCGCA AGTTGAATTA AAACCTATTC CTTACAGAAC    4157

CAAATGCATT AATAATTCTA GATATTTTTG GCCAAGACAA TCAGATTTTT CAATATTTCA    4217
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TATATACTAG GTGGAACACC ACCACCTGCA ACTCTGCAAC ACATGTTACG TTACACAATC 4277

ACTTTTGGCG GTTTTCAATT ATTTATATAA AATTGTAAAT GTTTGTACAC AGTAGAAAAT 4337

TAGTAATAGT GAATTTTGTT TCTCCGAATA TGTATAGCAA TATATATGGC ATGGATCAAA 4397

CTAGCCGACA TCCTAACTTG TTCACAGCTT TCCTTTTTAC TTATCTAGTC GATTAAGCAT 4457

CAGAAAGTAT GTTTTAATTT TTAAATTTGA AAAAGGTGTA CTTACAAGTT CGGGTGTTCA 4517

CACGGAGGAG AGCTACAATA ATGAAAAAGC TGACTCAAGA AGGGCTATAG AAGAAACAAG 4577

AGTCACGGAA CAAGTTGTCA CTCTCAATCT CCAGTACACT AGCTTCCATA ACTCTCTCTC 4637

TTTCTCTCTT TCTTCTCTCT CTAAAAGTTA TCAGAATAGA AATCTCTCTC TCTCAACAAG 4697

TCTAACAGTG CCATTTGTAT CTCTGAACTC CAACATGGCT CCTCTGGTTC TCTACCTTCT 4757

CACTCTCCTC ATGGCTGGCC ATTCCAGTAA GAACTCTCAC TGATCTTCTT CACCTTTGTT 4817

TATGGATTTG GTCTCTCAGT CTCACTCTCG CTTACCCTTT CACATTCAGC TCTGGCTCTC 4877

TGGTTTAAGA AACCCTTAAT CTACAAAGCT TGCTTTCCTC GCAAATGAAC TACCTTACTT 4937

ATCTCTTATG CAACTCTTGT TGATGATTTG CAAACATCTT AACCTCTCGA AACAAGATTT 4997

ACAAATCTTA CTGGCTTCAC TTACAATTTT GTTCCCATTT TTTTCTTCTT TGGTAGGTGC 5057

CTCATGGTGT GTGTGCAAAA CAGGGCTGAG TGACTCAGTG CTACAAAAGA CATTAGACTA 5117

TGCTTGTGGA AATGGAGCTG ACTGTAACCC AACTCACCCA AAAGGCTCTT GCTTCAATCC 5177

TGACAATGTT AGGGCTCATT GCAACTATGC AGTCAATAGC TTCTTCCAAA AGAAAGGTCA 5237

AGCTTCTGAG TCTTGTAACT TCACTGGTAC TGCCACTCTT ACCACCACCG ATCCCAGTAA 5297

GTTTTCAGAA TGTTAACACT CTTGTGATCT TTAGAACCCT ACAAAATTTT GAGTCTCAGA 5357

AAGTTCAAGT TCAAGGTCTT TTGGTTAGAG TACTAAAGAT TCAAGTAGAG ACTAGGCGTG 5417

AGATATTTTT TCTCTGATGT GTGATTTTTT GGCACAGGCT ATACAGGATG TGCATTCCCT 5477

TCTAGTGCTA GGTACGGCTC TTTGCTTCTC TACACATTTA TTTTCTTAAT GGCTTTATCT 5537

AGAACTTTGA AGGATACCAT TTTATTTTTT TTGGACAAAG AAGGATAGCC ATTTAATACT 5597

ACACTTTAAT GTTGGATTAA CTAACTTATT ATGCCTATTT AATGGCCTAC ACTTTAAGTG 5657

GACACAAGCT TGATTTGGTT ATAAAAAAAG TGCACTATAA TCTTATTTTA CTGAACCCTT 5717

TTTTCTATGA TTTTTTTACT AAACTTTAGA TAACATCTAC AACAATTCAA TTGCCTTTTT 5777

TTGGGGATTG TATAAGTTTG AACCTATGGT TAGTGTATTG ACTTGCGCGT CTCTTATTGC 5837

AACGGTTCTT TGAAAACACA TTAATGATAA ATAAATTGAA AAGTATAGAG ATGGCAATTG 5897

TTTCAAAAGC TAATCTTTCT GCTTGCTAAT ACTTTACATA AAAAACAAAA AATTAAGAAG 5957

ATTTTCAAAC AATACAACTT TTTTACCTTG TCCTAACAAA TTCAACTCAA ATGACATGTG 6017

TTTGCTTTAA AATAGTAACA ACTGTAAATT CATTTGCTCT TGAGACATAA GTGCAAGCTA 6077

AAGATAAACG CAAGCAATAC AATTAGGCCT AATTAAGATT ACGAATATTG TTGTTTGTTT 6137

ATAGTGGTTC TAGTGGAAGC GGTAGCACCA CCGTGACGCC AGGCAAAAAC AGTCCAAAAG 6197

GAAGCAACAG CATCACCACA TTTCCCGGCG GAAACAGTCC ATACACTGGC ACACCATCCA 6257

CCGGATTATT AGGAGGCAAT ATCACTGATG CAACTGGAAC CGGGTTGAAC CCGGATTACT 6317

CAACCGAAAG CAGTGGATTT GCGCTCTATT ACTCCAACAA CCTTCTGTTA ACCGGCTTTT 6377

GTTCTCTCGT GATGATGCTC TGAAGAAGAA TCACCGTCTT CTTTTAGTTT ATGCTTAGTC 6437

AAAAAAATAT GTTATTTATA TGTTCTTGTT GTTTTAGAGA TAATTTAATC TGGATTTCGG 6497

TTCTTTTTTA CTTTCCGGTT TTAAGAAAAC AATTATCAAT GTAAAACCAA ATCTACTATC 6557

GATCGGTTTG GTACGAATTC CTGCAGCCCG GGGGATCC 6595

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Thr Leu Phe Leu Thr Ile Leu Leu Ala Thr Val Leu Phe Leu
1 5 10 15

Ile Leu Arg Ile Phe Ser His Arg Arg Asn Arg Ser His Asn Asn Arg
20 25 30

Leu Pro Pro Gly Pro Asn Pro Trp Pro Ile Ile Gly Asn Leu Pro His
35 40 45

Met Gly Thr Lys Pro His Arg Thr Leu Ser Ala Met Val Thr Thr Tyr
50 55 60

Gly Pro Ile Leu His Leu Arg Leu Gly Phe Val Asp Val Val Val Ala
65 70 75 80

Ala Ser Lys Ser Val Ala Glu Gln Phe Leu Lys Ile His Asp Ala Asn
85 90 95

Phe Ala Ser Arg Pro Pro Asn Ser Gly Ala Lys His Met Ala Tyr Asn
100 105 110

Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly His Arg Trp Arg Leu Leu
115 120 125

Arg Lys Ile Ser Ser Val His Leu Phe Ser Ala Lys Ala Leu Glu Asp
130 135 140

Phe Lys His Val Arg Gln
145 150

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Glu Glu Val Gly Thr Leu Thr Arg Glu Leu Val Arg Val Gly Thr Lys
1 5 10 15

Pro Val Asn Leu Gly Gln Leu Val Asn Met Cys Val Val Asn Ala Leu
20 25 30

Gly Arg Glu Met Ile Gly Arg Arg Leu Phe Gly Ala Asp Ala Asp His
35 40 45

Lys Ala Asp Glu Phe Arg Ser Met Val Thr Glu Met Met Ala Leu Ala
50 55 60

Gly Val Phe Asn Ile Gly Asp Phe Val Pro Ser Leu Asp Trp Leu Asp
65 70 75 80

Leu Gln Gly Val Ala Gly Lys Met Lys Arg Leu His Lys Arg Phe Asp

85 90 95

Ala Phe Leu Ser Ser Ile Leu Lys Glu His Glu Met Asn Gly Gln Asp
100 105 110

Gln Lys His Thr Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly Thr
115 120 125

Asp Leu Asp Gly Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys Ala
130 135 140

Leu Leu Leu
145

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asn Met Phe Thr Ala Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp
1 5 10 15

Ala Ile Ala Glu Leu Ile Arg His Pro Asp Ile Met Val Lys Ala Gln
20 25 30

Glu Glu Leu Asp Ile Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser
35 40 45

Asp Ile Ala Gln Leu Pro Tyr Leu Gln
50 55

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 160 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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Ala Val Ile Lys Glu Asn Phe Arg Leu His Pro Pro Thr Pro Leu Ser
 1               5                  10                  15

Leu Pro His Ile Ala Ser Glu Ser Cys Glu Ile Asn Gly Tyr His Ile
            20                  25                  30

Pro Lys Gly Ser Thr Leu Leu Thr Asn Ile Trp Ala Ile Ala Arg Asp
        35                  40                  45

Pro Asp Gln Trp Ser Asp Pro Leu Ala Phe Lys Pro Glu Arg Phe Leu
    50                  55                  60

Pro Gly Gly Glu Lys Ser Gly Val Asp Val Lys Gly Ser Asp Phe Glu
65                  70                  75                  80

Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Leu Ser Leu
                85                  90                  95

Gly Leu Arg Thr Ile Gln Phe Leu Thr Ala Thr Leu Val Gln Gly Phe
            100                 105                 110

Asp Trp Glu Leu Ala Gly Gly Val Thr Pro Glu Lys Leu Asn Met Glu
        115                 120                 125

Glu Ser Tyr Gly Leu Thr Leu Gln Arg Ala Val Pro Leu Val Val His
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130 135 140

Pro Lys Pro Arg Leu Ala Pro Asn Val Tyr Gly Leu Gly Ser Gly
145 150 155

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1748 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 22..1563

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGTCGAGAAA GAAGAACAGC C ATG TTT CTC ATA GTA GTG ATC ACC TTC CTC 51
Met Phe Leu Ile Val Val Ile Thr Phe Leu
1 5 10

TTC GCC GTG TTT TTG TTC CGG CTT CTT TTC TCC GGC AAA TCC CAA CGC 99
Phe Ala Val Phe Leu Phe Arg Leu Leu Phe Ser Gly Lys Ser Gln Arg
15 20 25

CAC TCG CTC CCT CTC CCT CCT GGC CCC AAA CCA TGG CCG GTG GTT GGC 147
His Ser Leu Pro Leu Pro Pro Gly Pro Lys Pro Trp Pro Val Val Gly
30 35 40

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AAC TTA CCT CAC TTG GGC CCC TTC CCG CAC CAC TCC ATC GCG GAG TTG      195
Asn Leu Pro His Leu Gly Pro Phe Pro His His Ser Ile Ala Glu Leu
         45                  50                  55

GCG AAG AAA CAC GGG CCG CTC ATG CAC CTC CGC CTC GGC TAC GTT GAC      243
Ala Lys Lys His Gly Pro Leu Met His Leu Arg Leu Gly Tyr Val Asp
     60                  65                  70

GTA GTC GTG GCG GCA TCA GCA TCC GTA GCG GCC CAG TTC TTG AAG ACT      291
Val Val Val Ala Ala Ser Ala Ser Val Ala Ala Gln Phe Leu Lys Thr
 75                  80                  85                  90

CAC GAC GCC AAT TTC TCC AGC CGA CCG CCC AAC TCC GGC GCC AAG CAC      339
His Asp Ala Asn Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His
                 95                 100                 105

CTC GCC TAT AAC TAC CAG GAC CTC GTG TTC AGG CCG TAC GGT CCA CGG      387
Leu Ala Tyr Asn Tyr Gln Asp Leu Val Phe Arg Pro Tyr Gly Pro Arg
            110                 115                 120

TGG CGC ATG TTC CGG AAG ATC AGC TCC GTC CAT CTG TTC TCC GGC AAA      435
Trp Arg Met Phe Arg Lys Ile Ser Ser Val His Leu Phe Ser Gly Lys
        125                 130                 135

GCC TTG GAT GAT CTT AAA CAC GTC CGG CAG GAG GAG GTA AGT GTG CTA      483
Ala Leu Asp Asp Leu Lys His Val Arg Gln Glu Glu Val Ser Val Leu
    140                 145                 150

GCG CAT GCC TTG GCA AAT TCA GGG TCA AAG GTA GTG AAC CTG GCG CAA      531
Ala His Ala Leu Ala Asn Ser Gly Ser Lys Val Val Asn Leu Ala Gln
155                 160                 165                 170

CTG CTG AAC CTG TGC ACG GTC AAT GCT CTA GGA AGG GTG ATG GTA GGG      579
Leu Leu Asn Leu Cys Thr Val Asn Ala Leu Gly Arg Val Met Val Gly
                175                 180                 185

CGG AGG GTT TTC GGC GAC GGC AGC GGA GGC GAC GAT CCG AAG GCG GAC      627
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Arg Arg Val Phe Gly Asp Gly Ser Gly Gly Asp Asp Pro Lys Ala Asp
            190                 195                 200

GAG TTC AAA TCG ATG GTG GTG GAG ATG ATG GTG TTG GCA GGA GTG TTC      675
Glu Phe Lys Ser Met Val Val Glu Met Met Val Leu Ala Gly Val Phe
        205                 210                 215

AAC ATA GGT GAC TTC ATC CCC TCT CTC GAA TGG CTT GAC TTG CAA GGC      723
Asn Ile Gly Asp Phe Ile Pro Ser Leu Glu Trp Leu Asp Leu Gln Gly
    220                 225                 230

GTG GCG TCC AAG ATG AAG AAG CTC CAC AAG AGA TTC GAC GAC TTC TTG      771
Val Ala Ser Lys Met Lys Lys Leu His Lys Arg Phe Asp Asp Phe Leu
235                 240                 245                 250

ACA GCC ATT GTC GAG GAC CAC AAG AAG GGC TCC GGC ACG GCG GGG CAC      819
Thr Ala Ile Val Glu Asp His Lys Lys Gly Ser Gly Thr Ala Gly His
                255                 260                 265

GTC GAC ATG TTG ACC ACT CTG CTC TCG CTC AAG GAA GAC GCC GAC GGC      867
Val Asp Met Leu Thr Thr Leu Leu Ser Leu Lys Glu Asp Ala Asp Gly
            270                 275                 280

GAA GGA GGC AAG CTC ACC GAT ACT GAA ATC AAA GCT TTG CTT TTG AAC      915
Glu Gly Gly Lys Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn
        285                 290                 295

ATG TTC ACG GCT GGC ACT GAT ACG TCA TCG AGC ACG GTG GAA TGG GCA      963
Met Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala
    300                 305                 310

ATA GCT GAA CTC ATT CGG CAC CCT CAT ATG CTA GCG CGA GTT CAG AAA      1011
Ile Ala Glu Leu Ile Arg His Pro His Met Leu Ala Arg Val Gln Lys
315                 320                 325                 330

GAG CTT GAC GAT TTT GTT GGC CAT GAC CGA CTT GTG ACC GAA TCC GAC      1059
Glu Leu Asp Asp Phe Val Gly His Asp Arg Leu Val Thr Glu Ser Asp
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                    335                 340                 345

ATA CCC AAC CTC CCT TAC CTC CAA GCC GTG ATC AAG GAA ACG TTC CGA      1107
Ile Pro Asn Leu Pro Tyr Leu Gln Ala Val Ile Lys Glu Thr Phe Arg
            350                 355                 360

CTC CAC CCA TCC ACT CCT CTC TCG TTG CCT CGT ATG GCA GCC GAG AGT      1155
Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Met Ala Ala Glu Ser
        365                 370                 375

TGC GAA ATC AAC GGG TAC CAC ATC CCG AAA GGC TCC ACA CTC TTG GTC      1203
Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr Leu Leu Val
    380                 385                 390

AAT GTA TGG GCC ATA TCG CGT GAC CCG GCT GAA TGG GCC GAC CCA CTG      1251
Asn Val Trp Ala Ile Ser Arg Asp Pro Ala Glu Trp Ala Asp Pro Leu
395                 400                 405                 410

GAG TTC AAG CCC GAG AGG TTC CTG CCG GGG GGC GAA AAG CCT AAT GTT      1299
Glu Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val
                415                 420                 425

GAT ATT AGA GGA AAC GAT TTT GAA GTC ATA CCC TTC GGT GCC GGG CGA      1347
Asp Ile Arg Gly Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg
            430                 435                 440

AGA ATA TGT GCC GGG ATG AGC TTG GGC CTG CGT ATG GTC CAT TTA ATG      1395
Arg Ile Cys Ala Gly Met Ser Leu Gly Leu Arg Met Val His Leu Met
        445                 450                 455

ACT GCA ACA TTG GTC CAC GCA TTT AAT TGG GCC TTG GCT GAT GGG CTG      1443
Thr Ala Thr Leu Val His Ala Phe Asn Trp Ala Leu Ala Asp Gly Leu
    460                 465                 470

ACC GCT GAG AAG TTA AAC ATG GAT GAA GCA TAT GGG CTC ACT CTA CAA      1491
Thr Ala Glu Lys Leu Asn Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln
475                 480                 485                 490
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CGA GCT GCA CCG TTA ATG GTG CAC CCG CGC ACC AGG CTG GCC CCA CAG 1539
Arg Ala Ala Pro Leu Met Val His Pro Arg Thr Arg Leu Ala Pro Gln
495 500 505

GCA TAT AAA ACT TCA TCA TCT TAATTAGAGA GCTATGTTCT GGGTGTGCCC 1590
Ala Tyr Lys Thr Ser Ser Ser
510

GGTTTGATGT CTCCATGTTT TCTATTTAGG TTTAAATCTG TAAGATAAGG TGATTCTATG 1650

CTGAATCACA AAAGTTGCTA TCTAAATTCC ATGTCCAATG AAAACGTTCT TCTTCCCTTC 1710

TTATAATTTA TGAATACTTA TGATATAGGC GACAGCAA 1748

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Phe Leu Ile Val Val Ile Thr Phe Leu Phe Ala Val Phe Leu Phe
1 5 10 15

Arg Leu Leu Phe Ser Gly Lys Ser Gln Arg His Ser Leu Pro Leu Pro
20 25 30

Pro Gly Pro Lys Pro Trp Pro Val Val Gly Asn Leu Pro His Leu Gly
35 40 45

Pro Phe Pro His His Ser Ile Ala Glu Leu Ala Lys Lys His Gly Pro
50 55 60

Leu Met His Leu Arg Leu Gly Tyr Val Asp Val Val Val Ala Ala Ser
65 70 75 80

Ala Ser Val Ala Ala Gln Phe Leu Lys Thr His Asp Ala Asn Phe Ser
85 90 95

Ser Arg Pro Pro Asn Ser Gly Ala Lys His Leu Ala Tyr Asn Tyr Gln
100 105 110

Asp Leu Val Phe Arg Pro Tyr Gly Pro Arg Trp Arg Met Phe Arg Lys
115 120 125

Ile Ser Ser Val His Leu Phe Ser Gly Lys Ala Leu Asp Asp Leu Lys
130 135 140

His Val Arg Gln Glu Glu Val Ser Val Leu Ala His Ala Leu Ala Asn
145 150 155 160

Ser Gly Ser Lys Val Val Asn Leu Ala Gln Leu Leu Asn Leu Cys Thr
165 170 175

Val Asn Ala Leu Gly Arg Val Met Val Gly Arg Arg Val Phe Gly Asp
180 185 190

Gly Ser Gly Gly Asp Asp Pro Lys Ala Asp Glu Phe Lys Ser Met Val
195 200 205

Val Glu Met Met Val Leu Ala Gly Val Phe Asn Ile Gly Asp Phe Ile
210 215 220

Pro Ser Leu Glu Trp Leu Asp Leu Gln Gly Val Ala Ser Lys Met Lys
225 230 235 240

Lys Leu His Lys Arg Phe Asp Asp Phe Leu Thr Ala Ile Val Glu Asp
245 250 255

His Lys Lys Gly Ser Gly Thr Ala Gly His Val Asp Met Leu Thr Thr

260 265 270

Leu Leu Ser Leu Lys Glu Asp Ala Asp Gly Glu Gly Gly Lys Leu Thr
275 280 285

Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Met Phe Thr Ala Gly Thr
290 295 300

Asp Thr Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg
305 310 315 320

His Pro His Met Leu Ala Arg Val Gln Lys Glu Leu Asp Asp Phe Val
325 330 335

Gly His Asp Arg Leu Val Thr Glu Ser Asp Ile Pro Asn Leu Pro Tyr
340 345 350

Leu Gln Ala Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro
355 360 365

Leu Ser Leu Pro Arg Met Ala Ala Glu Ser Cys Glu Ile Asn Gly Tyr
370 375 380

His Ile Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ser
385 390 395 400

Arg Asp Pro Ala Glu Trp Ala Asp Pro Leu Glu Phe Lys Pro Glu Arg
405 410 415

Phe Leu Pro Gly Gly Glu Lys Pro Asn Val Asp Ile Arg Gly Asn Asp
420 425 430

Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met
435 440 445

Ser Leu Gly Leu Arg Met Val His Leu Met Thr Ala Thr Leu Val His
450 455 460

Ala Phe Asn Trp Ala Leu Ala Asp Gly Leu Thr Ala Glu Lys Leu Asn
465 470 475 480

Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ala Pro Leu Met
485 490 495

Val His Pro Arg Thr Arg Leu Ala Pro Gln Ala Tyr Lys Thr Ser Ser
500 505 510

Ser

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1660 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4..1528

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AAA ATG ACC ATT TTA GCT TTC GTA TTT TAC GCC CTC ATC CTC GGG TCA 48
Met Thr Ile Leu Ala Phe Val Phe Tyr Ala Leu Ile Leu Gly Ser
1 5 10 15

GTA CTC TAT GTA TTT CTT AAC TTA AGT TCA CGT AAA TCC GCC AGA CTC 96

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Val Leu Tyr Val Phe Leu Asn Leu Ser Ser Arg Lys Ser Ala Arg Leu
                 20                  25                  30

CCA CCC GGG CCA ACA CCA TGG CCT ATA GTC GGG AAC TTA CCA CAC CTT      144
Pro Pro Gly Pro Thr Pro Trp Pro Ile Val Gly Asn Leu Pro His Leu
            35                  40                  45

GGC CCA ATC CCA CAC CAC GCA CTC GCG GCC TTA GCC AAG AAG TAC GGG      192
Gly Pro Ile Pro His His Ala Leu Ala Ala Leu Ala Lys Lys Tyr Gly
        50                  55                  60

CCA TTG ATG CAC CTG CGG CTC GGG TGT GTG GAC GTG GTT GTG GCC GCG      240
Pro Leu Met His Leu Arg Leu Gly Cys Val Asp Val Val Val Ala Ala
    65                  70                  75

TCT GCT TCC GTA GCT GCA CAG TTT TTA AAA GTT CAC GAC GCA AAT TTT      288
Ser Ala Ser Val Ala Ala Gln Phe Leu Lys Val His Asp Ala Asn Phe
80                  85                  90                  95

GCT AGT AGG CCG CCA AAT TCT GGC GCG AAA CAT GTG GCG TAT AAT TAT      336
Ala Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr
                100                 105                 110

CAG GAT CTT GTG TTT GCA CCT TAT GGT CCA AGG TGG CGT TTG TTA AGG      384
Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Leu Leu Arg
            115                 120                 125

AAG ATT TGT TCG GTC CAT TTG TTT TCT GCT AAA GCA CTT GAT GAT TTT      432
Lys Ile Cys Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe
        130                 135                 140

CGT CAT GTT CGA CAG GAG GAG GTA GCA GTC CTA ACC CGC GTA CTA CTG      480
Arg His Val Arg Gln Glu Glu Val Ala Val Leu Thr Arg Val Leu Leu
    145                 150                 155

AGT GCT GGA AAC TCA CCG GTA CAG CTT GGC CAA CTA CTT AAC GTG TGT      528
Ser Ala Gly Asn Ser Pro Val Gln Leu Gly Gln Leu Leu Asn Val Cys
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160 165 170 175

GCC ACA AAC GCC TTA GCA CGG GTA ATG TTA GGT AGG AGA GTT TTC GGA 576
Ala Thr Asn Ala Leu Ala Arg Val Met Leu Gly Arg Arg Val Phe Gly
180 185 190

GAC GGA ATT GAC AGG TCA GCC AAT GAG TTC AAA GAT ATG GTA GTA GAG 624
Asp Gly Ile Asp Arg Ser Ala Asn Glu Phe Lys Asp Met Val Val Glu
195 200 205

TTA ATG GTA TTA GCA GGA GAA TTT AAC CTT GGT GAC TTT ATT CCT GTA 672
Leu Met Val Leu Ala Gly Glu Phe Asn Leu Gly Asp Phe Ile Pro Val
210 215 220

CTT GAC CTA TTC GAC CTA CAA GGC ATT ACT AAA AAA ATG AAG AAG CTT 720
Leu Asp Leu Phe Asp Leu Gln Gly Ile Thr Lys Lys Met Lys Lys Leu
225 230 235

CAT GTT CGG TTC GAT TCA TTT CTT AGT AAG ATC GTT GAG GAG CAT AAA 768
His Val Arg Phe Asp Ser Phe Leu Ser Lys Ile Val Glu Glu His Lys
240 245 250 255

ACG GCA CCT GGT GGG TTG GGT CAT ACT GAT TTG CTG AGC ACG TTG ATT 816
Thr Ala Pro Gly Gly Leu Gly His Thr Asp Leu Leu Ser Thr Leu Ile
260 265 270

TCA CTT AAA GAT GAT GCT GAT ATT GAA GGT GGG AAG CTT ACA GAT ACT 864
Ser Leu Lys Asp Asp Ala Asp Ile Glu Gly Gly Lys Leu Thr Asp Thr
275 280 285

GAA ATC AAA GCT TTG CTT CTG AAT TTA TTC GCT GCG GGA ACA GAC ACA 912
Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ala Ala Gly Thr Asp Thr
290 295 300

TCC TCT AGT ACA GTA GAA TGG GCA ATA GCC GAA CTC ATT CGT CAT CCA 960
Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg His Pro
305 310 315

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CAA ATA TTA AAA CAA GCC CGA GAA GAG ATA GAC GCT GTA GTT GGT CAA      1008
Gln Ile Leu Lys Gln Ala Arg Glu Glu Ile Asp Ala Val Val Gly Gln
320                 325                 330                 335

GAC CGG CTT GTA ACA GAA TTG GAC TTG AGC CAA CTA ACA TAC CTC CAG      1056
Asp Arg Leu Val Thr Glu Leu Asp Leu Ser Gln Leu Thr Tyr Leu Gln
                340                 345                 350

GCT CTT GTG AAA GAG GTG TTT AGG CTC CAC CCT TCA ACG CCA CTC TCC      1104
Ala Leu Val Lys Glu Val Phe Arg Leu His Pro Ser Thr Pro Leu Ser
            355                 360                 365

TTA CCA AGA ATA TCA TCC GAG AGT TGT GAG GTC GAT GGG TAT TAT ATC      1152
Leu Pro Arg Ile Ser Ser Glu Ser Cys Glu Val Asp Gly Tyr Tyr Ile
        370                 375                 380

CCT AAG GGA TCC ACA CTC CTC GTT AAC GTG TGG GCC ATT GCG CGA GAC      1200
Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp
    385                 390                 395

CCA AAA ATG TGG GCG GAT CCT CTT GAA TTT AGG CCT TCT CGG TTT TTA      1248
Pro Lys Met Trp Ala Asp Pro Leu Glu Phe Arg Pro Ser Arg Phe Leu
400                 405                 410                 415

CCC GGG GGA GAA AAG CCC GGT GCT GAT GTT AGG GGA AAT GAT TTT GAA      1296
Pro Gly Gly Glu Lys Pro Gly Ala Asp Val Arg Gly Asn Asp Phe Glu
                420                 425                 430

GTT ATA CCA TTT GGG GCA GGA CGA AGG ATT TGT GCG GGT ATG AGC CTA      1344
Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu
            435                 440                 445

GGC TTG AGA ATG GTC CAG TTG CTC ATT GCA ACA TTG GTC CAA ACT TTT      1392
Gly Leu Arg Met Val Gln Leu Leu Ile Ala Thr Leu Val Gln Thr Phe
        450                 455                 460

GAT TGG GAA CTG GCT AAC GGG TTA GAG CCG GAG ATG CTC AAC ATG GAA      1440
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Asp Trp Glu Leu Ala Asn Gly Leu Glu Pro Glu Met Leu Asn Met Glu
465 470 475

GAA GCG TAT GGA TTG ACC CTT CAA CGG GCT GCA CCC TTG ATG GTT CAC 1488
Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ala Pro Leu Met Val His
480 485 490 495

CCG AAG CCG AGG TTA GCT CCC CAC GTA TAT GAA AGT ATT T AAGGACTAGT 1538
Pro Lys Pro Arg Leu Ala Pro His Val Tyr Glu Ser Ile
500 505

TTCTCTTTTG CCTTTTTGTT TCGCAAAGGT TAATGAATAA ACGATTTCAT GACTCAGATA 1598

GTTATGTAAA CAATTGTGTT TGCTGTTTAT ATATTTATCT ATTTTTCTAG AACAAAAAAA 1658

AA 1660

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 508 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Thr Ile Leu Ala Phe Val Phe Tyr Ala Leu Ile Leu Gly Ser Val
1 5 10 15

Leu Tyr Val Phe Leu Asn Leu Ser Ser Arg Lys Ser Ala Arg Leu Pro
20 25 30

Pro Gly Pro Thr Pro Trp Pro Ile Val Gly Asn Leu Pro His Leu Gly
35 40 45

Pro Ile Pro His His Ala Leu Ala Ala Leu Ala Lys Lys Tyr Gly Pro
50 55 60

Leu Met His Leu Arg Leu Gly Cys Val Asp Val Val Val Ala Ala Ser
65 70 75 80

Ala Ser Val Ala Ala Gln Phe Leu Lys Val His Asp Ala Asn Phe Ala
85 90 95

Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr Gln
100 105 110

Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Leu Leu Arg Lys
115 120 125

Ile Cys Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe Arg
130 135 140

His Val Arg Gln Glu Glu Val Ala Val Leu Thr Arg Val Leu Leu Ser
145 150 155 160

Ala Gly Asn Ser Pro Val Gln Leu Gly Gln Leu Leu Asn Val Cys Ala
165 170 175

Thr Asn Ala Leu Ala Arg Val Met Leu Gly Arg Arg Val Phe Gly Asp
180 185 190

Gly Ile Asp Arg Ser Ala Asn Glu Phe Lys Asp Met Val Val Glu Leu
195 200 205

Met Val Leu Ala Gly Glu Phe Asn Leu Gly Asp Phe Ile Pro Val Leu
210 215 220

Asp Leu Phe Asp Leu Gln Gly Ile Thr Lys Lys Met Lys Lys Leu His
225 230 235 240

Val Arg Phe Asp Ser Phe Leu Ser Lys Ile Val Glu Glu His Lys Thr

245 250 255

Ala Pro Gly Gly Leu Gly His Thr Asp Leu Leu Ser Thr Leu Ile Ser
260 265 270

Leu Lys Asp Asp Ala Asp Ile Glu Gly Gly Lys Leu Thr Asp Thr Glu
275 280 285

Ile Lys Ala Leu Leu Leu Asn Leu Phe Ala Ala Gly Thr Asp Thr Ser
290 295 300

Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg His Pro Gln
305 310 315 320

Ile Leu Lys Gln Ala Arg Glu Glu Ile Asp Ala Val Val Gly Gln Asp
325 330 335

Arg Leu Val Thr Glu Leu Asp Leu Ser Gln Leu Thr Tyr Leu Gln Ala
340 345 350

Leu Val Lys Glu Val Phe Arg Leu His Pro Ser Thr Pro Leu Ser Leu
355 360 365

Pro Arg Ile Ser Ser Glu Ser Cys Glu Val Asp Gly Tyr Tyr Ile Pro
370 375 380

Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp Pro
385 390 395 400

Lys Met Trp Ala Asp Pro Leu Glu Phe Arg Pro Ser Arg Phe Leu Pro
405 410 415

Gly Gly Glu Lys Pro Gly Ala Asp Val Arg Gly Asn Asp Phe Glu Val
420 425 430

Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu Gly
435 440 445

Leu Arg Met Val Gln Leu Leu Ile Ala Thr Leu Val Gln Thr Phe Asp
450 455 460

Trp Glu Leu Ala Asn Gly Leu Glu Pro Glu Met Leu Asn Met Glu Glu
465 470 475 480

Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ala Pro Leu Met Val His Pro
485 490 495

Lys Pro Arg Leu Ala Pro His Val Tyr Glu Ser Ile
500 505

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1815 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 107..1631

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTAAATTAAT TAATAAATAC ACACACGACG AGATGTATGT AATGTAATGT AATATTATTA 60

CATACATCAT CACCGAATAC GCACGCTACT ACCACTGCGA TTAGCC ATG AGT CCC 115
Met Ser Pro
1

TTA GCC TTG ATG ATC ATA AGT ACC TTA TTA GGG TTT CTC CTA TAC CAC 163

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Leu Ala Leu Met Ile Ile Ser Thr Leu Leu Gly Phe Leu Leu Tyr His
      5                  10                  15

TCT CTT CGC TTA CTA CTC TTC TCC GGC CAA GGT CGC CGA CTA CTA CCA      211
Ser Leu Arg Leu Leu Leu Phe Ser Gly Gln Gly Arg Arg Leu Leu Pro
 20                  25                  30                  35

CCA GGT CCA CGC CCG TGG CCG CTG GTG GGA AAT CTC CCG CAC TTA GGC      259
Pro Gly Pro Arg Pro Trp Pro Leu Val Gly Asn Leu Pro His Leu Gly
                 40                  45                  50

CCG AAG CCA CAC GCC TCC ATG GCC GAG CTC GCG CGA GCC TAC GGA CCC      307
Pro Lys Pro His Ala Ser Met Ala Glu Leu Ala Arg Ala Tyr Gly Pro
             55                  60                  65

CTC ATG CAC CTA AAG ATG GGG TTC GTC CAC GTC GTG GTG GCT TCG TCG      355
Leu Met His Leu Lys Met Gly Phe Val His Val Val Val Ala Ser Ser
         70                  75                  80

GCG AGC GCG GCG GAG CAG TGC CTG AGG GTT CAC GAC GCG AAT TTC TTG      403
Ala Ser Ala Ala Glu Gln Cys Leu Arg Val His Asp Ala Asn Phe Leu
     85                  90                  95

AGC AGG CCA CCC AAC TCC GGC GCC AAG CAC GTC GCT TAC AAC TAC GAG      451
Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr Glu
100                 105                 110                 115

GAC TTG GTT TTC AGA CCG TAC GGT CCC AAG TGG AGG CTG TTG AGG AAG      499
Asp Leu Val Phe Arg Pro Tyr Gly Pro Lys Trp Arg Leu Leu Arg Lys
                120                 125                 130

ATA TGC GCT CAG CAT ATT TTC TCC GTC AAG GCT ATG GAT GAC TTC AGG      547
Ile Cys Ala Gln His Ile Phe Ser Val Lys Ala Met Asp Asp Phe Arg
            135                 140                 145

CGC GTC AGA GAG GAA GAG GTG GCC ATC CTG AGT CGC GCT CTA GCA GGC      595
Arg Val Arg Glu Glu Glu Val Ala Ile Leu Ser Arg Ala Leu Ala Gly
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150 155 160

AAA AGG GCC GTA CCC ATA GGC CAA ATG CTC AAC GTG TGC GCC ACA AAC 643
Lys Arg Ala Val Pro Ile Gly Gln Met Leu Asn Val Cys Ala Thr Asn
165 170 175

GCC CTA TCT CGC GTC ATG ATG GGG CGG CGC GTG GTG GGC CAC GCG GAT 691
Ala Leu Ser Arg Val Met Met Gly Arg Arg Val Val Gly His Ala Asp
180 185 190 195

GGA ACC AAC GAC GCC AAG GCG GAG GAG TTC AAA GCC ATG GTC GTC GAG 739
Gly Thr Asn Asp Ala Lys Ala Glu Glu Phe Lys Ala Met Val Val Glu
200 205 210

CTC ATG GTC CTC TCC GGC GTC TTC AAC ATC GGT GAT TTC ATC CCC TTC 787
Leu Met Val Leu Ser Gly Val Phe Asn Ile Gly Asp Phe Ile Pro Phe
215 220 225

CTC GAG CCT CTC GAC TTG CAG GGA GTG GCT TCC AAG ATG AAG AAA CTC 835
Leu Glu Pro Leu Asp Leu Gln Gly Val Ala Ser Lys Met Lys Lys Leu
230 235 240

CAC GCG CGG TTC GAT GCA TTC TTG ACC GAG ATT GTA CGA GAG CGT TGT 883
His Ala Arg Phe Asp Ala Phe Leu Thr Glu Ile Val Arg Glu Arg Cys
245 250 255

CAT GGG CAG ATC AAC AAC AGT GGT GCT CAT CAG GAT GAT TTG CTT AGC 931
His Gly Gln Ile Asn Asn Ser Gly Ala His Gln Asp Asp Leu Leu Ser
260 265 270 275

ACG TTG ATT TCG TTC AAA GGG CTT GAC GAT GGC GAT GGT TCC AGG CTC 979
Thr Leu Ile Ser Phe Lys Gly Leu Asp Asp Gly Asp Gly Ser Arg Leu
280 285 290

ACT GAC ACA GAA ATC AAG GCG CTG CTC TTG AAC CTT TTG GAC ACG ACG 1027
Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Leu Asp Thr Thr
295 300 305

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TCG AGC ACG GTG GAA TGG GCC GTA GCC GAA CTC CTA CGC CAC CCT AAG      1075
Ser Ser Thr Val Glu Trp Ala Val Ala Glu Leu Leu Arg His Pro Lys
        310                 315                 320

ACA TTA GCC CAA GTC CGG CAA GAG CTC GAC TCG GTC GTG GGT AAG AAC      1123
Thr Leu Ala Gln Val Arg Gln Glu Leu Asp Ser Val Val Gly Lys Asn
    325                 330                 335

AGG CTC GTG TCC GAG ACC GAT CTG AAT CAG CTG CCC TAT CTA CAA GCT      1171
Arg Leu Val Ser Glu Thr Asp Leu Asn Gln Leu Pro Tyr Leu Gln Ala
340                 345                 350                 355

GTC GTC AAA GAA ACT TTC CGC CTC CAT CCT CCG ACG CCG CTC TCT CTA      1219
Val Val Lys Glu Thr Phe Arg Leu His Pro Pro Thr Pro Leu Ser Leu
                360                 365                 370

CCG AGA CTC GCG GAA GAT GAT TGC GAG ATC GAC GGA TAC CTC ATC CCC      1267
Pro Arg Leu Ala Glu Asp Asp Cys Glu Ile Asp Gly Tyr Leu Ile Pro
            375                 380                 385

AAG GGC TCG ACC CTT CTG GTG AAC GTT TGG GCC ATA GCC CGC GAT CCC      1315
Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp Pro
        390                 395                 400

AAG GTT TGG GCC GAT CCG TTG GAG TTT AGG CCC GAA CGA TTC TTG ACG      1363
Lys Val Trp Ala Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu Thr
    405                 410                 415

GGC GGA GAA AAG GCC GAC GTC GAT GTC AAG GGG AAC GAT TTC GAA GTG      1411
Gly Gly Glu Lys Ala Asp Val Asp Val Lys Gly Asn Asp Phe Glu Val
420                 425                 430                 435

ATA CCG TTC GGG GCG GGT CGT AGG ATC TGC GCT GGC GTT GGC TTG GGA      1459
Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Val Gly Leu Gly
                440                 445                 450

ATA CGT ATG GTC CAA CTG TTG ACG GCG AGT TTG ATC CAT GCA TTC GAT      1507
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Ile Arg Met Val Gln Leu Leu Thr Ala Ser Leu Ile His Ala Phe Asp
455 460 465

CTG GAC CTT GCT AAT GGG CTT TTG GCC CAA AAT CTG AAC ATG GAA GAA 1555
Leu Asp Leu Ala Asn Gly Leu Leu Ala Gln Asn Leu Asn Met Glu Glu
470 475 480

GCA TAT GGG CTT ACG CTA CAA CGG GCT GAG CCT TTG TTG GTC CAC CCT 1603
Ala Tyr Gly Leu Thr Leu Gln Arg Ala Glu Pro Leu Leu Val His Pro
485 490 495

AGG CCG CGG TTG GCC ACT CAT GTC TAT T AATTAAATTA GGCCTAAACT 1651
Arg Pro Arg Leu Ala Thr His Val Tyr
500 505

ACGATGAATG ACCCATTTAA CGTTAATAAG AGTTTTCAAT TTATGTGAGT TTGCATGGTA 1711

TGGTATGGTA TGGTGCTTGT AATAAATTGT ATCTGTTAGG TGTGTTCATT GATGATAAAT 1771

CTAGTTTGTA CTGCTGCTCA AAAAAAAAAA AAAAAAAAAA AAAA 1815

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 508 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ser Pro Leu Ala Leu Met Ile Ile Ser Thr Leu Leu Gly Phe Leu
1 5 10 15

Leu Tyr His Ser Leu Arg Leu Leu Leu Phe Ser Gly Gln Gly Arg Arg

20 25 30

Leu Leu Pro Pro Gly Pro Arg Pro Trp Pro Leu Val Gly Asn Leu Pro
35 40 45

His Leu Gly Pro Lys Pro His Ala Ser Met Ala Glu Leu Ala Arg Ala
50 55 60

Tyr Gly Pro Leu Met His Leu Lys Met Gly Phe Val His Val Val Val
65 70 75 80

Ala Ser Ser Ala Ser Ala Ala Glu Gln Cys Leu Arg Val His Asp Ala
85 90 95

Asn Phe Leu Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr
100 105 110

Asn Tyr Glu Asp Leu Val Phe Arg Pro Tyr Gly Pro Lys Trp Arg Leu
115 120 125

Leu Arg Lys Ile Cys Ala Gln His Ile Phe Ser Val Lys Ala Met Asp
130 135 140

Asp Phe Arg Arg Val Arg Glu Glu Glu Val Ala Ile Leu Ser Arg Ala
145 150 155 160

Leu Ala Gly Lys Arg Ala Val Pro Ile Gly Gln Met Leu Asn Val Cys
165 170 175

Ala Thr Asn Ala Leu Ser Arg Val Met Met Gly Arg Arg Val Val Gly
180 185 190

His Ala Asp Gly Thr Asn Asp Ala Lys Ala Glu Glu Phe Lys Ala Met
195 200 205

Val Val Glu Leu Met Val Leu Ser Gly Val Phe Asn Ile Gly Asp Phe
210 215 220

Ile Pro Phe Leu Glu Pro Leu Asp Leu Gln Gly Val Ala Ser Lys Met
225 230 235 240

Lys Lys Leu His Ala Arg Phe Asp Ala Phe Leu Thr Glu Ile Val Arg
245 250 255

Glu Arg Cys His Gly Gln Ile Asn Asn Ser Gly Ala His Gln Asp Asp
260 265 270

Leu Leu Ser Thr Leu Ile Ser Phe Lys Gly Leu Asp Asp Gly Asp Gly
275 280 285

Ser Arg Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Leu
290 295 300

Asp Thr Thr Ser Ser Thr Val Glu Trp Ala Val Ala Glu Leu Leu Arg
305 310 315 320

His Pro Lys Thr Leu Ala Gln Val Arg Gln Glu Leu Asp Ser Val Val
325 330 335

Gly Lys Asn Arg Leu Val Ser Glu Thr Asp Leu Asn Gln Leu Pro Tyr
340 345 350

Leu Gln Ala Val Val Lys Glu Thr Phe Arg Leu His Pro Pro Thr Pro
355 360 365

Leu Ser Leu Pro Arg Leu Ala Glu Asp Asp Cys Glu Ile Asp Gly Tyr
370 375 380

Leu Ile Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala
385 390 395 400

Arg Asp Pro Lys Val Trp Ala Asp Pro Leu Glu Phe Arg Pro Glu Arg
405 410 415

Phe Leu Thr Gly Gly Glu Lys Ala Asp Val Asp Val Lys Gly Asn Asp

420 425 430

Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Val
435 440 445

Gly Leu Gly Ile Arg Met Val Gln Leu Leu Thr Ala Ser Leu Ile His
450 455 460

Ala Phe Asp Leu Asp Leu Ala Asn Gly Leu Leu Ala Gln Asn Leu Asn
465 470 475 480

Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Glu Pro Leu Leu
485 490 495

Val His Pro Arg Pro Arg Leu Ala Thr His Val Tyr
500 505

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1824 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..1553

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

G AGC TTA ACC TTA ATT TTC TGC ACT TTA GTT TTT GCA ATC TTT CTA 46
Ser Leu Thr Leu Ile Phe Cys Thr Leu Val Phe Ala Ile Phe Leu

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    1               5                  10                  15

TAT TTT CTT ATT CTC AGG GTG AAA CAG CGT TAC CCT TTA CCT CTC CCA      94
Tyr Phe Leu Ile Leu Arg Val Lys Gln Arg Tyr Pro Leu Pro Leu Pro
                20                  25                  30

CCC GGA CCA AAA CCA TGG CCG GTG TTA GGA AAC CTT CCC CAC CTG GGC     142
Pro Gly Pro Lys Pro Trp Pro Val Leu Gly Asn Leu Pro His Leu Gly
            35                  40                  45

AAG AAG CCT CAC CAG TCG ATT GCG GCC ATG GCT GAG AGG TAC GGC CCC     190
Lys Lys Pro His Gln Ser Ile Ala Ala Met Ala Glu Arg Tyr Gly Pro
        50                  55                  60

CTC ATG CAC CTC CGC CTA GGA TTC GTG GAC GTG GTT GTG GCC GCC TCC     238
Leu Met His Leu Arg Leu Gly Phe Val Asp Val Val Val Ala Ala Ser
    65                  70                  75

GCC GCC GTG GCC GCT CAG TTC TTG AAA GTT CAC GAC TCG AAC TTC TCC     286
Ala Ala Val Ala Ala Gln Phe Leu Lys Val His Asp Ser Asn Phe Ser
80                  85                  90                  95

AAC CGG CCG CCG AAC TCC GGC GCG GAA CAC ATT GCT TAT AAC TAT CAA     334
Asn Arg Pro Pro Asn Ser Gly Ala Glu His Ile Ala Tyr Asn Tyr Gln
                100                 105                 110

GAC CTC GTC TTC GCG CCC TAC GGC CCG CGG TGG CGC ATG CTT AGG AAG     382
Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys
            115                 120                 125

ATC ACC TCC GTG CAT CTC TTC TCG GCC AAG GCG TTG GAT GAC TTC TGC     430
Ile Thr Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe Cys
        130                 135                 140

CAT GTT CGC CAG GAA GAG GTT GCA ACT CTG ACA CGC AGT CTA GCA AGT     478
His Val Arg Gln Glu Glu Val Ala Thr Leu Thr Arg Ser Leu Ala Ser
    145                 150                 155
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GCA GGC AAA ACT CCA GTA AAA CTA GGG CAG TTA CTA AAC GTG TGC ACC      526
Ala Gly Lys Thr Pro Val Lys Leu Gly Gln Leu Leu Asn Val Cys Thr
160                 165                 170                 175

ACG AAC GCC CTA GCT CGT GTA ATG CTA GGG CGG AAG GTC TTT AAT GAC      574
Thr Asn Ala Leu Ala Arg Val Met Leu Gly Arg Lys Val Phe Asn Asp
                180                 185                 190

GGA GGT AGC AAG AGC GAC CCA AAG GCG GAG GAG TTC AAG TCG ATG GTG      622
Gly Gly Ser Lys Ser Asp Pro Lys Ala Glu Glu Phe Lys Ser Met Val
            195                 200                 205

GAG GAG ATG ATG GTG TTG GCC GGA AGT TTT AAC ATC GGC GAT TTC ATT      670
Glu Glu Met Met Val Leu Ala Gly Ser Phe Asn Ile Gly Asp Phe Ile
        210                 215                 220

CCG GTC TTG GGT TGG TTT GAC GTT CAG GGT ATC GTA GGG AAG ATG AAG      718
Pro Val Leu Gly Trp Phe Asp Val Gln Gly Ile Val Gly Lys Met Lys
    225                 230                 235

AAA CTA CAC GCG CGT TTT GAT GCG TTC TTG AAC ACC ATT CTA GAG GAA      766
Lys Leu His Ala Arg Phe Asp Ala Phe Leu Asn Thr Ile Leu Glu Glu
240                 245                 250                 255

CAC AAA TGT GTC AAC AAT CAA CAC ACG ACG TTG TCG AAA GAT GTG GAC      814
His Lys Cys Val Asn Asn Gln His Thr Thr Leu Ser Lys Asp Val Asp
                260                 265                 270

TTC TTG AGC ACC CTA ATT AGG CTC AAA GAT AAT GGG GCT GAT ATG GAT      862
Phe Leu Ser Thr Leu Ile Arg Leu Lys Asp Asn Gly Ala Asp Met Asp
            275                 280                 285

TGT GAA GAG GGA AAA CTC ACC GAC ACT GAA ATT AAG GCT TTG CTC TTG      910
Cys Glu Glu Gly Lys Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu
        290                 295                 300

AAC CTG TTC ACA GCT GGG ACT GAT ACA TCA TCT AGC ACT GTG GAG TGG      958
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Asn Leu Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp
305                 310                 315

GCA ATC GCA GAA CTA CTA CGC AAC CCA AAA ATC TTA AAC CAA GCA CAA      1006
Ala Ile Ala Glu Leu Leu Arg Asn Pro Lys Ile Leu Asn Gln Ala Gln
320                 325                 330                 335

CAA GAG CTT GAC TTA GTG GTG GGT CAA AAT CAG CTA GTC ACA GAA TCT      1054
Gln Glu Leu Asp Leu Val Val Gly Gln Asn Gln Leu Val Thr Glu Ser
                340                 345                 350

GAC TTA ACC GAT CTA CCT TTC CTG CAA GCA ATA GTG AAG GAG ACC TTC      1102
Asp Leu Thr Asp Leu Pro Phe Leu Gln Ala Ile Val Lys Glu Thr Phe
            355                 360                 365

AGG CTA CAC CCA TCC ACC CCA CTC TCT CTT CCA AGA ATG GGA GCT CAG      1150
Arg Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Met Gly Ala Gln
        370                 375                 380

GGT TGC GAG ATC AAT GGC TAC TTC ATC CCC AAA GGC GCA ACG CTT TTG      1198
Gly Cys Glu Ile Asn Gly Tyr Phe Ile Pro Lys Gly Ala Thr Leu Leu
385                 390                 395

GTC AAC GTT TGG GCC ATA GCT CGT GAT CCC AAT GTG TGG ACA AAT CCT      1246
Val Asn Val Trp Ala Ile Ala Arg Asp Pro Asn Val Trp Thr Asn Pro
400                 405                 410                 415

CTT GAG TTC AAC CCA CAC CGA TTC TTG CCT GGT GGA GAA AAG CCC AAC      1294
Leu Glu Phe Asn Pro His Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn
                420                 425                 430

GTG GAT ATT AAA GGG AAT GAC TTT GAA GTG ATT CCT TTT GGA GCC GGG      1342
Val Asp Ile Lys Gly Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly
            435                 440                 445

CGT AGA ATA TGC TCT GGG ATG AGT TTG GGG ATA AGG ATG GTT CAC CTG      1390
Arg Arg Ile Cys Ser Gly Met Ser Leu Gly Ile Arg Met Val His Leu
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450 455 460

TTG GTT GCA ACT TTG GTG CAT GCT TTT GAT TGG GAT TTG GTG AAT GGA 1438
Leu Val Ala Thr Leu Val His Ala Phe Asp Trp Asp Leu Val Asn Gly
465 470 475

CAA TCT GTA GAG ACG CTC AAT ATG GAG GAA GCT TAT GGT CTC ACC CTT 1486
Gln Ser Val Glu Thr Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu
480 485 490 495

CAA CGA GCT GTT CCT TTG ATG TTG CAT CCA AAG CCC AGA TTA CAA CCA 1534
Gln Arg Ala Val Pro Leu Met Leu His Pro Lys Pro Arg Leu Gln Pro
500 505 510

CAT CTC TAT ACT CTC AAT T AAATTGCAAT TTGATTTTGG TGATTATACA 1583
His Leu Tyr Thr Leu Asn
515

ATTATAATCG AGGGACATAG GATCCCCATT TATTTATATT CAGTTATAAG AGACTTCCAA 1643

CAAAGGTCTA GCTTTCGACC TTAAAAGTTG TAAAAGAGGT CCTACATATG TAAAAGCCCG 1703

CCAAAGGAAA ACTGGTTGTA TTCAATTCCG CTAGGCCTTG TCCGAAAGAC CTCATGAAGA 1763

CTACAAAGGT CATATATAAT GGTAAACCCA GTGTATTTGT TGTAAAAAAA AAAAAAAAAA 1823

A 1824

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 517 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Leu Thr Leu Ile Phe Cys Thr Leu Val Phe Ala Ile Phe Leu Tyr
1 5 10 15

Phe Leu Ile Leu Arg Val Lys Gln Arg Tyr Pro Leu Pro Leu Pro Pro
20 25 30

Gly Pro Lys Pro Trp Pro Val Leu Gly Asn Leu Pro His Leu Gly Lys
35 40 45

Lys Pro His Gln Ser Ile Ala Ala Met Ala Glu Arg Tyr Gly Pro Leu
50 55 60

Met His Leu Arg Leu Gly Phe Val Asp Val Val Val Ala Ala Ser Ala
65 70 75 80

Ala Val Ala Ala Gln Phe Leu Lys Val His Asp Ser Asn Phe Ser Asn
85 90 95

Arg Pro Pro Asn Ser Gly Ala Glu His Ile Ala Tyr Asn Tyr Gln Asp
100 105 110

Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Ile
115 120 125

Thr Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe Cys His
130 135 140

Val Arg Gln Glu Glu Val Ala Thr Leu Thr Arg Ser Leu Ala Ser Ala
145 150 155 160

Gly Lys Thr Pro Val Lys Leu Gly Gln Leu Leu Asn Val Cys Thr Thr
165 170 175

Asn Ala Leu Ala Arg Val Met Leu Gly Arg Lys Val Phe Asn Asp Gly
180 185 190

Gly Ser Lys Ser Asp Pro Lys Ala Glu Glu Phe Lys Ser Met Val Glu
195 200 205

Glu Met Met Val Leu Ala Gly Ser Phe Asn Ile Gly Asp Phe Ile Pro
210 215 220

Val Leu Gly Trp Phe Asp Val Gln Gly Ile Val Gly Lys Met Lys Lys
225 230 235 240

Leu His Ala Arg Phe Asp Ala Phe Leu Asn Thr Ile Leu Glu Glu His
245 250 255

Lys Cys Val Asn Asn Gln His Thr Thr Leu Ser Lys Asp Val Asp Phe
260 265 270

Leu Ser Thr Leu Ile Arg Leu Lys Asp Asn Gly Ala Asp Met Asp Cys
275 280 285

Glu Glu Gly Lys Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn
290 295 300

Leu Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala
305 310 315 320

Ile Ala Glu Leu Leu Arg Asn Pro Lys Ile Leu Asn Gln Ala Gln Gln
325 330 335

Glu Leu Asp Leu Val Val Gly Gln Asn Gln Leu Val Thr Glu Ser Asp
340 345 350

Leu Thr Asp Leu Pro Phe Leu Gln Ala Ile Val Lys Glu Thr Phe Arg
355 360 365

Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Met Gly Ala Gln Gly
370 375 380

Cys Glu Ile Asn Gly Tyr Phe Ile Pro Lys Gly Ala Thr Leu Leu Val

385 390 395 400

Asn Val Trp Ala Ile Ala Arg Asp Pro Asn Val Trp Thr Asn Pro Leu
405 410 415

Glu Phe Asn Pro His Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val
420 425 430

Asp Ile Lys Gly Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg
435 440 445

Arg Ile Cys Ser Gly Met Ser Leu Gly Ile Arg Met Val His Leu Leu
450 455 460

Val Ala Thr Leu Val His Ala Phe Asp Trp Asp Leu Val Asn Gly Gln
465 470 475 480

Ser Val Glu Thr Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln
485 490 495

Arg Ala Val Pro Leu Met Leu His Pro Lys Pro Arg Leu Gln Pro His
500 505 510

Leu Tyr Thr Leu Asn
515

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1667 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1429

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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CCC ATC CTC GGA AAC ATC CCC CAT CTC GGC TCC AAA CCG CAC CAA ACA      48
Pro Ile Leu Gly Asn Ile Pro His Leu Gly Ser Lys Pro His Gln Thr
 1               5                  10                  15

CTC GCG GAA ATG GCG AAA ACC TAC GGT CCG CTC ATG CAC TTG AAG TTC      96
Leu Ala Glu Met Ala Lys Thr Tyr Gly Pro Leu Met His Leu Lys Phe
            20                  25                  30

GGG CTT AAG GAC GCG GTG GTG GCG TCG TCT GCG TCG GTG GCA GAG CAG     144
Gly Leu Lys Asp Ala Val Val Ala Ser Ser Ala Ser Val Ala Glu Gln
        35                  40                  45

TTT CTG AAG AAA CAC GAC GTG AAT TTC TCG AAC CGG CCG CCA AAC TCC     192
Phe Leu Lys Lys His Asp Val Asn Phe Ser Asn Arg Pro Pro Asn Ser
    50                  55                  60

GGG GCC AAA CAT ATA GCT TAT AAC TAT CAG GAC CTG GTA TTC GCT CCC     240
Gly Ala Lys His Ile Ala Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro
65                  70                  75                  80

TAT GGA CCC CGG TGG CGG TTG CTT AGG AAA ATC TGT TCC GTC CAT CTT     288
Tyr Gly Pro Arg Trp Arg Leu Leu Arg Lys Ile Cys Ser Val His Leu
                85                  90                  95

TTC TCG TCT AAG GCC TTG GAT GAC TTT CAG CAT GTT CGA CAT GAG GAG     336
Phe Ser Ser Lys Ala Leu Asp Asp Phe Gln His Val Arg His Glu Glu
            100                 105                 110

ATA TGC ATC CTT ATA CGA GCA ATA GCG AGT GGC GGT CAT GCT CCG GTG     384
Ile Cys Ile Leu Ile Arg Ala Ile Ala Ser Gly Gly His Ala Pro Val
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115 120 125

AAT TTA GGC AAG TTA TTA GGA GTG TGC ACA ACC AAT GCC CTG GCA AGA 432
Asn Leu Gly Lys Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg
130 135 140

GTG ATG CTT GGA AGA AGA GTA TTC GAA GGC GAC GGC GGC GAG AAT CCG 480
Val Met Leu Gly Arg Arg Val Phe Glu Gly Asp Gly Gly Glu Asn Pro
145 150 155 160

CAT GCC GAC GAG TTT AAA TCA ATG GTG GTG GAG ATT ATG GTG TTA GCC 528
His Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu Ala
165 170 175

GGT GCA TTC AAC TTG GGT GAT TTC ATC CCG GTT CTA GAT TGG TTC GAT 576
Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Val Leu Asp Trp Phe Asp
180 185 190

TTG CAA GGA ATT GCT GGT AAA ATG AAG AAA CTT CAT GCC CGT TTC GAC 624
Leu Gln Gly Ile Ala Gly Lys Met Lys Lys Leu His Ala Arg Phe Asp
195 200 205

AAG TTT TTA AAT GGG ATC CTA GAA GAT CGT AAA TCT AAC GGC TCT AAT 672
Lys Phe Leu Asn Gly Ile Leu Glu Asp Arg Lys Ser Asn Gly Ser Asn
210 215 220

GGA GCT GAA CAA TAC GTG GAC TTG CTC AGT GTG TTG ATC TCT CTT CAA 720
Gly Ala Glu Gln Tyr Val Asp Leu Leu Ser Val Leu Ile Ser Leu Gln
225 230 235 240

GAT AGT AAT ATC GAC GGT GGT GAC GAA GGA ACC AAA CTC ACA GAT ACT 768
Asp Ser Asn Ile Asp Gly Gly Asp Glu Gly Thr Lys Leu Thr Asp Thr
245 250 255

GAA ATC AAA GCT CTC CTT TTG AAC TTG TTC ATA GCC GGA ACA GAC ACT 816
Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ile Ala Gly Thr Asp Thr
260 265 270

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TCA TCA AGT ACT GTA GAA TGG GCC ATG GCA GAA CTA ATC CGA AAC CCA      864
Ser Ser Ser Thr Val Glu Trp Ala Met Ala Glu Leu Ile Arg Asn Pro
        275                 280                 285

AAG TTA CTA GTC CAA GCC CAA GAA GAG CTA GAC AGA GTA GTC GGG CCG      912
Lys Leu Leu Val Gln Ala Gln Glu Glu Leu Asp Arg Val Val Gly Pro
    290                 295                 300

AAC CGA TTC GTA ACC GAA TCT GAT CTT CCT CAA CTG ACA TTC CTT CAA      960
Asn Arg Phe Val Thr Glu Ser Asp Leu Pro Gln Leu Thr Phe Leu Gln
305                 310                 315                 320

GCC GTC ATC AAA GAG ACT TTC AGG CTT CAT CCA TCC ACC CCA CTC TCT     1008
Ala Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro Leu Ser
                325                 330                 335

CTT CCA CGA ATG GCG GCG GAG GAC TGT GAG ATC AAT GGG TAT TAT GTC     1056
Leu Pro Arg Met Ala Ala Glu Asp Cys Glu Ile Asn Gly Tyr Tyr Val
            340                 345                 350

TCA GAA GGT TCG ACA TTG CTC GTC AAT GTG TGG GCC ATA GCT CGT GAT     1104
Ser Glu Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp
        355                 360                 365

CCA AAT GCG TGG GCC AAT CCA CTA GAT TTC AAC CCG ACT CGT TTC TTG     1152
Pro Asn Ala Trp Ala Asn Pro Leu Asp Phe Asn Pro Thr Arg Phe Leu
    370                 375                 380

GCC GGT GGA GAG AAG CCT AAT GTT GAT GTT AAA GGG AAT GAT TTT GAA     1200
Ala Gly Gly Glu Lys Pro Asn Val Asp Val Lys Gly Asn Asp Phe Glu
385                 390                 395                 400

GTG ATA CCT TTC GGT GCT GGG CGC AGG ATA TGT GCC GGA ATG AGC TTA     1248
Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu
                405                 410                 415

GGT ATA CGG ATG GTT CAA CTA GTA ACG GCT TCG TTA GTT CAT TCG TTT     1296
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Gly Ile Arg Met Val Gln Leu Val Thr Ala Ser Leu Val His Ser Phe
420 425 430

GAT TGG GCT TTG TTG GAT GGA CTT AAA CCC GAG AAG CTT GAC ATG GAG 1344
Asp Trp Ala Leu Leu Asp Gly Leu Lys Pro Glu Lys Leu Asp Met Glu
435 440 445

GAA GGT TAT GGA CTA ACG CTT CAA CGA GCT TCA CCT TTA ATC GTC CAT 1392
Glu Gly Tyr Gly Leu Thr Leu Gln Arg Ala Ser Pro Leu Ile Val His
450 455 460

CCA AAG CCG AGG CTC TCG GCT CAA GTT TAT TGT ATG T AACAAGTTTG 1439
Pro Lys Pro Arg Leu Ser Ala Gln Val Tyr Cys Met
465 470 475

TGAAGCCAGT CTGATTTCAG TTGGATTTGT AGTTATTTTA TGATCATTTG GTATTTTATT 1499

TTGTATTTCG GTTGAATACA ATAAAGGGAA GGTGGATCGT CTGCTGTATA ATAGCGACGT 1559

TTTAACGTGT TGTGATAGTA CCGTGTTTTA CTAAAACGAT GTCGTTTGAT TTTTTATAGT 1619

ATTAAAAAAA TAAACAGCTG GATTTTGAAC CAAAAAAAAA AAAAAAAA 1667

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 476 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Pro Ile Leu Gly Asn Ile Pro His Leu Gly Ser Lys Pro His Gln Thr
1 5 10 15

Leu Ala Glu Met Ala Lys Thr Tyr Gly Pro Leu Met His Leu Lys Phe
20 25 30

Gly Leu Lys Asp Ala Val Val Ala Ser Ser Ala Ser Val Ala Glu Gln
35 40 45

Phe Leu Lys Lys His Asp Val Asn Phe Ser Asn Arg Pro Pro Asn Ser
50 55 60

Gly Ala Lys His Ile Ala Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro
65 70 75 80

Tyr Gly Pro Arg Trp Arg Leu Leu Arg Lys Ile Cys Ser Val His Leu
85 90 95

Phe Ser Ser Lys Ala Leu Asp Asp Phe Gln His Val Arg His Glu Glu
100 105 110

Ile Cys Ile Leu Ile Arg Ala Ile Ala Ser Gly Gly His Ala Pro Val
115 120 125

Asn Leu Gly Lys Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg
130 135 140

Val Met Leu Gly Arg Arg Val Phe Glu Gly Asp Gly Gly Glu Asn Pro
145 150 155 160

His Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu Ala
165 170 175

Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Val Leu Asp Trp Phe Asp
180 185 190

Leu Gln Gly Ile Ala Gly Lys Met Lys Lys Leu His Ala Arg Phe Asp
195 200 205

Lys Phe Leu Asn Gly Ile Leu Glu Asp Arg Lys Ser Asn Gly Ser Asn

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     210                 215                 220

Gly Ala Glu Gln Tyr Val Asp Leu Leu Ser Val Leu Ile Ser Leu Gln
225                 230                 235                 240

Asp Ser Asn Ile Asp Gly Gly Asp Glu Gly Thr Lys Leu Thr Asp Thr
                245                 250                 255

Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ile Ala Gly Thr Asp Thr
            260                 265                 270

Ser Ser Ser Thr Val Glu Trp Ala Met Ala Glu Leu Ile Arg Asn Pro
        275                 280                 285

Lys Leu Leu Val Gln Ala Gln Glu Glu Leu Asp Arg Val Val Gly Pro
    290                 295                 300

Asn Arg Phe Val Thr Glu Ser Asp Leu Pro Gln Leu Thr Phe Leu Gln
305                 310                 315                 320

Ala Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro Leu Ser
                325                 330                 335

Leu Pro Arg Met Ala Ala Glu Asp Cys Glu Ile Asn Gly Tyr Tyr Val
            340                 345                 350

Ser Glu Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp
        355                 360                 365

Pro Asn Ala Trp Ala Asn Pro Leu Asp Phe Asn Pro Thr Arg Phe Leu
    370                 375                 380

Ala Gly Gly Glu Lys Pro Asn Val Asp Val Lys Gly Asn Asp Phe Glu
385                 390                 395                 400

Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu
                405                 410                 415
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Gly Ile Arg Met Val Gln Leu Val Thr Ala Ser Leu Val His Ser Phe
420 425 430

Asp Trp Ala Leu Leu Asp Gly Leu Lys Pro Glu Lys Leu Asp Met Glu
435 440 445

Glu Gly Tyr Gly Leu Thr Leu Gln Arg Ala Ser Pro Leu Ile Val His
450 455 460

Pro Lys Pro Arg Leu Ser Ala Gln Val Tyr Cys Met
465 470 475

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1214 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..1091

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

T CGC ATC CTC ACG CGA TCT ATA GCG AGT GCT GGG GAA AAT CCG ATT 46
Arg Ile Leu Thr Arg Ser Ile Ala Ser Ala Gly Glu Asn Pro Ile
1 5 10 15

AAC TTA GGT CAA TTA CTC GGG GTG TGT ACC ACA AAT GCT CTG GCG AGA 94
Asn Leu Gly Gln Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg
20 25 30

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GTG ATG CTT GGA AGG AGG GTA TTC GGC GAT GGG AGC GGC GGC GTA GAT      142
Val Met Leu Gly Arg Arg Val Phe Gly Asp Gly Ser Gly Gly Val Asp
             35                  40                  45

CCT CAG GCG GAC GAG TTC AAA TCC ATG GTG GTG GAA ATC ATG GTG TTG      190
Pro Gln Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu
         50                  55                  60

GCC GGC GCG TTT AAT CTA GGT GAT TTT ATT CCC GCT CTT GAT TGG TTC      238
Ala Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Ala Leu Asp Trp Phe
     65                  70                  75

GAT CTG CAG GGA ATT ACG GCA AAA ATG AAG AAA GTT CAC GCT CGT TTC      286
Asp Leu Gln Gly Ile Thr Ala Lys Met Lys Lys Val His Ala Arg Phe
 80                  85                  90                  95

GAT GCG TTC TTA GAC GCG ATC CTT GAG GAG CAC AAA TCC AAC GGC TCT      334
Asp Ala Phe Leu Asp Ala Ile Leu Glu Glu His Lys Ser Asn Gly Ser
                100                 105                 110

CGC GGA GCT AAG CAA CAC GTT GAC TTG CTG AGT ATG TTG ATC TCC CTT      382
Arg Gly Ala Lys Gln His Val Asp Leu Leu Ser Met Leu Ile Ser Leu
            115                 120                 125

CAA GAT AAT AAC ATT GAT GGT GAA AGT GGC GCC AAA CTC ACT GAT ACA      430
Gln Asp Asn Asn Ile Asp Gly Glu Ser Gly Ala Lys Leu Thr Asp Thr
        130                 135                 140

GAA ATC AAA GCT TTG CTT CTG AAC TTG TTC ACG GCT GGA ACA GAC ACG      478
Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Thr Ala Gly Thr Asp Thr
    145                 150                 155

TCA TCA AGT ACT GTG GAG TGG GCA ATC GCA GAG CTA ATC CGA AAC CCA      526
Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg Asn Pro
160                 165                 170                 175

GAA GTA TTG GTT CAA GCC CAA CAA GAG CTC GAT AGA GTA GTT GGG CCA      574
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Glu Val Leu Val Gln Ala Gln Gln Glu Leu Asp Arg Val Val Gly Pro
                180                 185                 190

AGT CGT CTT GTG ACC GAA TCT GAT CTG CCT CAA TTG GCA TTC CTT CAA      622
Ser Arg Leu Val Thr Glu Ser Asp Leu Pro Gln Leu Ala Phe Leu Gln
            195                 200                 205

GCT GTC ATC AAA GAG ACT TTC AGA CTT CAT CCA TCC ACT CCA CTC TCT      670
Ala Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro Leu Ser
        210                 215                 220

CTT CCA CGA ATG GCT TCA GAG GGT TGT GAA ATC AAT GGA TAC TCC ATC      718
Leu Pro Arg Met Ala Ser Glu Gly Cys Glu Ile Asn Gly Tyr Ser Ile
    225                 230                 235

CCA AAG GGT TCG ACA TTG CTC GTT AAC GTA TGG TCC ATA GCC CGT GAT      766
Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ser Ile Ala Arg Asp
240                 245                 250                 255

CCT AGT ATA TGG GCC GAC CCA TTA GAA TTT AGG CCG GCA CGT TTC TTG      814
Pro Ser Ile Trp Ala Asp Pro Leu Glu Phe Arg Pro Ala Arg Phe Leu
                260                 265                 270

CCC GGC GGA GAA AAG CCC AAT GTT GAT GTG AGA GGC AAT GAT TTT GAG      862
Pro Gly Gly Glu Lys Pro Asn Val Asp Val Arg Gly Asn Asp Phe Glu
            275                 280                 285

GTC ATA CCA TTT GGT GCT GGA CGT AGG ATA TGT GCT GGA ATG AGC TTG      910
Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu
        290                 295                 300

GGT TTA AGA ATG GTT CAA CTT TCG ACA GCT ACT TTG GTT CAT TCG TTT      958
Gly Leu Arg Met Val Gln Leu Ser Thr Ala Thr Leu Val His Ser Phe
    305                 310                 315

AAT TGG GAT TTG CTG AAT GGG ATG AGC CCA GAT AAA CTT GAC ATG GAA      1006
Asn Trp Asp Leu Leu Asn Gly Met Ser Pro Asp Lys Leu Asp Met Glu
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320 325 330 335

GAA GCT TAT GGG CTT ACA TTG CAA CGG GCT TCA CCT TTG ATT GTC CAC 1054
Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ser Pro Leu Ile Val His
340 345 350

CCA AAG CCC AGG CTT GCT AGC TCT ATG TAT GTT AAA T GAAATTATGC 1101
Pro Lys Pro Arg Leu Ala Ser Ser Met Tyr Val Lys
355 360

TGTGCGAATA ATTCCTTATT TATAGCAGGA AATGTCATCT TGAATTATGT GTAATGTTCT 1161

TCTAACTTTC GATGGAAGTG CAAAACAAGT TTTATTAAAA AAAAAAAAAA AAA 1214

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 363 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Arg Ile Leu Thr Arg Ser Ile Ala Ser Ala Gly Glu Asn Pro Ile Asn
1 5 10 15

Leu Gly Gln Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg Val
20 25 30

Met Leu Gly Arg Arg Val Phe Gly Asp Gly Ser Gly Gly Val Asp Pro
35 40 45

Gln Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu Ala
50 55 60

Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Ala Leu Asp Trp Phe Asp
65 70 75 80

Leu Gln Gly Ile Thr Ala Lys Met Lys Lys Val His Ala Arg Phe Asp
85 90 95

Ala Phe Leu Asp Ala Ile Leu Glu Glu His Lys Ser Asn Gly Ser Arg
100 105 110

Gly Ala Lys Gln His Val Asp Leu Leu Ser Met Leu Ile Ser Leu Gln
115 120 125

Asp Asn Asn Ile Asp Gly Glu Ser Gly Ala Lys Leu Thr Asp Thr Glu
130 135 140

Ile Lys Ala Leu Leu Leu Asn Leu Phe Thr Ala Gly Thr Asp Thr Ser
145 150 155 160

Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg Asn Pro Glu
165 170 175

Val Leu Val Gln Ala Gln Gln Glu Leu Asp Arg Val Val Gly Pro Ser
180 185 190

Arg Leu Val Thr Glu Ser Asp Leu Pro Gln Leu Ala Phe Leu Gln Ala
195 200 205

Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro Leu Ser Leu
210 215 220

Pro Arg Met Ala Ser Glu Gly Cys Glu Ile Asn Gly Tyr Ser Ile Pro
225 230 235 240

Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ser Ile Ala Arg Asp Pro
245 250 255

Ser Ile Trp Ala Asp Pro Leu Glu Phe Arg Pro Ala Arg Phe Leu Pro

260 265 270

Gly Gly Glu Lys Pro Asn Val Asp Val Arg Gly Asn Asp Phe Glu Val
275 280 285

Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu Gly
290 295 300

Leu Arg Met Val Gln Leu Ser Thr Ala Thr Leu Val His Ser Phe Asn
305 310 315 320

Trp Asp Leu Leu Asn Gly Met Ser Pro Asp Lys Leu Asp Met Glu Glu
325 330 335

Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ser Pro Leu Ile Val His Pro
340 345 350

Lys Pro Arg Leu Ala Ser Ser Met Tyr Val Lys
355 360

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1757 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 35..1522

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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CCGTTGCTGT CGAGAAAACA GAAAGAAGAG AAAA ATG GAC TAC GTG AAT ATT          52
                                       Met Asp Tyr Val Asn Ile
                                        1               5

TTG CTG GGA CTG TTT TTC ACT TGG TTC TTG GTG AAT GGA CTC ATG TCA        100
Leu Leu Gly Leu Phe Phe Thr Trp Phe Leu Val Asn Gly Leu Met Ser
            10                  15                  20

CTT CGA AGA AGA AAA ATC TCT AAG AAA CTT CCA CCA GGT CCA TTT CCT        148
Leu Arg Arg Arg Lys Ile Ser Lys Lys Leu Pro Pro Gly Pro Phe Pro
        25                  30                  35

TTG CCT ATC ATC GGA AAT CTT CAC TTA CTT GGT AAT CAT CCT CAC AAA        196
Leu Pro Ile Ile Gly Asn Leu His Leu Leu Gly Asn His Pro His Lys
    40                  45                  50

TCA CTT GCT CAA CTT GCA AAA ATT CAT GGT CCT ATT ATG AAT CTC AAA        244
Ser Leu Ala Gln Leu Ala Lys Ile His Gly Pro Ile Met Asn Leu Lys
55                  60                  65                  70

TTA GGC CAA CTA AAC ACA GTG GTC ATT TCA TCA TCA GTC GTG GCA AGA        292
Leu Gly Gln Leu Asn Thr Val Val Ile Ser Ser Ser Val Val Ala Arg
                75                  80                  85

GAA GTC TTG CAA AAA CAA GAC TTA ACA TTT TCC AAT AGG TTT GTC CCG        340
Glu Val Leu Gln Lys Gln Asp Leu Thr Phe Ser Asn Arg Phe Val Pro
            90                  95                  100

GAC GTA GTC CAT GTC CGA AAT CAC TCC GAT TTT TCT GTT GTT TGG TTA        388
Asp Val Val His Val Arg Asn His Ser Asp Phe Ser Val Val Trp Leu
        105                 110                 115

CCA GTC AAT TCT CGA TGG AAA ACG CTT CGC AAA ATC ATG AAC TCT AGC        436
Pro Val Asn Ser Arg Trp Lys Thr Leu Arg Lys Ile Met Asn Ser Ser
    120                 125                 130

ATC TTT TCT GGT AAC AAG CTT GAT GGT AAT CAA CAT CTG AGG TCT AAA        484
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Ile Phe Ser Gly Asn Lys Leu Asp Gly Asn Gln His Leu Arg Ser Lys
135                 140                 145                 150

AAG GTC CAA GAG TTA ATT GAT TAT TGT CAA AAG TGT GCC AAG AAT GGC      532
Lys Val Gln Glu Leu Ile Asp Tyr Cys Gln Lys Cys Ala Lys Asn Gly
                155                 160                 165

GAA GCA GTG GAT ATA GGA AGA GCA ACT TTT GGA ACT ACT TTG AAT TTG      580
Glu Ala Val Asp Ile Gly Arg Ala Thr Phe Gly Thr Thr Leu Asn Leu
            170                 175                 180

CTA TCC AAC ACC ATT TTC TCT AAA GAT TTG ACT AAT CCG TTT TCT GAT      628
Leu Ser Asn Thr Ile Phe Ser Lys Asp Leu Thr Asn Pro Phe Ser Asp
        185                 190                 195

TCT GCT AAA GAG TTT AAG GAA TTG GTT TGG AAC ATT ATG GTT GAG GCT      676
Ser Ala Lys Glu Phe Lys Glu Leu Val Trp Asn Ile Met Val Glu Ala
    200                 205                 210

GGA AAA CCC AAT TTG GTG GAC TAC TTT CCT TTC CTT GAG AAA ATT GAT      724
Gly Lys Pro Asn Leu Val Asp Tyr Phe Pro Phe Leu Glu Lys Ile Asp
215                 220                 225                 230

CCG CAA GGT ATA AAG CGA CGC ATG ACT AAT AAT TTT ACT AAG TTT CTT      772
Pro Gln Gly Ile Lys Arg Arg Met Thr Asn Asn Phe Thr Lys Phe Leu
                235                 240                 245

GGC CTT ATC AGC GGT TTG ATT GAT GAC CGG TTA AAG GAA AGG AAT CTA      820
Gly Leu Ile Ser Gly Leu Ile Asp Asp Arg Leu Lys Glu Arg Asn Leu
            250                 255                 260

AGG GAC AAT GCA AAT ATT GAT GTT TTA GAC GCC CTT CTC AAC ATT AGC      868
Arg Asp Asn Ala Asn Ile Asp Val Leu Asp Ala Leu Leu Asn Ile Ser
        265                 270                 275

CAA GAG AAC CCA GAA GAG ATT GAC AGG AAT CAA ATC GAG CAG TTG TGT      916
Gln Glu Asn Pro Glu Glu Ile Asp Arg Asn Gln Ile Glu Gln Leu Cys
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280 285 290

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CTG GAC TTG TTT GCA GCA GGG ACT GAT ACT ACA TCG AAT ACC TTG GAG      964
Leu Asp Leu Phe Ala Ala Gly Thr Asp Thr Thr Ser Asn Thr Leu Glu
295                 300                 305                 310

TGG GCA ATG GCA GAA CTA CTT CAG AAT CCA CAC ACA TTG CAG AAA GCA     1012
Trp Ala Met Ala Glu Leu Leu Gln Asn Pro His Thr Leu Gln Lys Ala
                315                 320                 325

CAA GAA GAA CTT GCA CAA GTC ATT GGT AAA GGC AAA CAA GTA GAA GAA     1060
Gln Glu Glu Leu Ala Gln Val Ile Gly Lys Gly Lys Gln Val Glu Glu
            330                 335                 340

GCA GAT GTT GGA CGA CTA CCT TAC TTG CGA TGC ATA GTG AAA GAA ACC     1108
Ala Asp Val Gly Arg Leu Pro Tyr Leu Arg Cys Ile Val Lys Glu Thr
        345                 350                 355

TTA CGA ATA CAC CCA GCG GCT CCT CTC TTA ATT CCA CGT AAA GTG GAG     1156
Leu Arg Ile His Pro Ala Ala Pro Leu Leu Ile Pro Arg Lys Val Glu
    360                 365                 370

GAA GAC GTT GAG TTG TCT ACC TAT ATT ATT CCA AAG GAT TCA CAA GTT     1204
Glu Asp Val Glu Leu Ser Thr Tyr Ile Ile Pro Lys Asp Ser Gln Val
375                 380                 385                 390

CTA GTG AAC GTA TGG GCA ATT GGA CGC AAC TCT GAT CTA TGG GAA AAT     1252
Leu Val Asn Val Trp Ala Ile Gly Arg Asn Ser Asp Leu Trp Glu Asn
                395                 400                 405

CCT TTG GTC TTT AAG CCA GAA AGG TTT TGG GAG TCA GAA ATA GAT ATC     1300
Pro Leu Val Phe Lys Pro Glu Arg Phe Trp Glu Ser Glu Ile Asp Ile
            410                 415                 420

CGA GGT CGA GAT TTT GAA CTC ATT CCA TTT GGT GCT GGT CGA AGA ATT     1348
Arg Gly Arg Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile
        425                 430                 435
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TGC CCT GGA TTG CCT TTG GCT ATG AGG ATG ATT CCA GTA GCA CTA GGT      1396
Cys Pro Gly Leu Pro Leu Ala Met Arg Met Ile Pro Val Ala Leu Gly
    440                 445                 450

TCA TTG CTA AAC TCA TTT AAT TGG AAA CTA TAT GGT GGA ATT GCA CCT      1444
Ser Leu Leu Asn Ser Phe Asn Trp Lys Leu Tyr Gly Gly Ile Ala Pro
455                 460                 465                 470

AAA GAT TTG GAC ATG CAG GAA AAG TTT GGC ATT ACC TTG GCG AAA GCC      1492
Lys Asp Leu Asp Met Gln Glu Lys Phe Gly Ile Thr Leu Ala Lys Ala
                475                 480                 485

CAA CCT CTG CTA GCT ATC CCA ACT CCC CTG TAGCTATAGG GATAAATTAA        1542
Gln Pro Leu Leu Ala Ile Pro Thr Pro Leu
            490                 495

GTTGAGGTTT TAAGTTACTA GTAGATTCTA TTGCAGCTAT AGGATTTCTT TCACCATCAC    1602

GTATGCTTTA CCGTTGGATG ATGGAAAGAA ATATCTATAG CTTTGGGTTT GTTTAGTTTG    1662

CACATAAAAA TTGAATGAAT GGAATACCAT GGAGTTATAA GAAATAATAA GACTATGATT    1722

CTTACCCTAC TTGAACAATG ACATGGCTAT TTCAC                               1757
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(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TTTTTTTTTT TTTTTTTA 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTTTTTTTTT TTTTTTTC

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTTTTTTTTT TTTTTTTG 18

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Trp Ala Ile Gly Arg Asp Pro
5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGGGCIATIG GI(A/C)GIGA(T/C)CC 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Phe Arg Pro Glu Arg Phe
5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 acids

(B) TYPE: nucleic acids

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AGGAATT(T/C)(A/C)G ICCIGA(A/G)(A/C)GI TT 22

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 nucleic acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCITT(T/C)GGIG CIGGI(A/C)GI(A/C)G IATITG(T/G)(C/G)CI GG 32

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Phe Xaa Pro Glu Arg Phe
5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleic acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAITT(T/C)IIIC CIGAI(A/C)GITT 20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 nucleic acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCACACGAGT AGTTTTGGCA TTTGACCC 28

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 nucleic acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GTCTTGGACA TCACACTTCA ATCTG 25

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 nucleic acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CCGAATTCCC CCCCCCC 17

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCIGG(A/G)CAIA TIC(G/T)(C/T)(C/T)TICC IGCICC(A/G)AAI GG 32

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding a flavonoid 3′-hydroxylase or a derivative thereof wherein said flavonoid 3′-hydroxylase or its derivative is capable of more efficient modulation of hydroxylation of flavonoid compounds in plants than is a flavonoid 3′-hydroxylase encoded by the nucleotide sequence set forth in SEQ ID NO:26.

2. An isolated nucleic acid molecule according to claim 1 comprising a nucleotide sequence which corresponds to the genetic locus designated *Ht1* or *Ht2* in petunia or to loci in other flowering plant species which contain sequences which control production of 3′-hydroxylated flavonoids.

3. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:1 under low stringency conditions.

4. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:3 under low stringency conditions.

5. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:5 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:5 under low stringency conditions.

6. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:7

or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:7 under low stringency conditions.

7. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:9 or having at least about 60% similarity to the coding region thereof or capable of hybridizing to the sequence set forth in SEQ ID NO:9 under low stringency conditions.

8. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:14 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:14 under low stringency conditions.

9. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:16 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:16 under low stringency conditions.

10. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:18 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:18 under low stringency conditions.

11. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:20 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:20 under low stringency conditions.

12. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:22

or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:22 under low stringency conditions.

13. An isolated nucleic acid molecule according to claim 2 comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:24 or having at least about 60% similarity thereto or capable of hybridizing to the sequence set forth in SEQ ID NO:24 under low stringency conditions.

14. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least about 50% similarity thereto.

15. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least about 50% similarity thereto.

16. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity thereto.

17. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

18. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence

substantially as set forth in SEQ ID NO:10 or SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13 or an amino acid sequence having at least about 50% similarity thereto.

19. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:15 or an amino acid sequence having at least about 50% similarity thereto.

20. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:17 or an amino acid sequence having at least about 50% similarity thereto.

21. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:19 or an amino acid sequence having at least about 50% similarity thereto.

22. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:21 or an amino acid sequence having at least about 50% similarity thereto.

23. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:23 or an amino acid sequence having at least about 50% similarity thereto.

24. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence

substantially as set forth in SEQ ID NO:25 or an amino acid sequence having at least about 50% similarity thereto.

25. An oligonucleotide capable of hybridizing under low stringency conditions to a nucleotide sequence selected from SEQ ID NO:1, 3, 5, 7, 9, 14, 16, 18, 20, 22 and 24.

26. A genetic construct capable of reducing expression of an endogenous gene encoding a flavonoid 3′-hydroxylase in a plant, said genetic construct comprising a nucleotide sequence selected from:

(i) a nucleotide sequence encoding an amino acid sequence set forth in one of SEQ ID NO:2, 4, 6, 8, 10, 11, 12, 13, 15, 17, 19, 21, 23 or 25 or a complementary form thereof;

(ii) a nucleotide sequence set forth in one of SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22 or 24 or the coding region in SEQ ID NO:9 or a complementary form thereof;

(iii) a nucleotide sequence having at least about 60% similarity to (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to (i), (ii) and/or (iii).

27. A method for producing a transgenic plant capable of synthesizing a flavonoid 3′-hydroxylase or a functional derivative thereof, said method comprising stably transforming a cell of a suitable plant with nucleic acid molecule which comprises a sequence of nucleotides encoding said flavonoid 3′-hydroxylase or a derivative thereof under conditions permitting the eventual expression of said nucleic acid molecule, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid molecule.

28. A method for producing a transgenic plant with reduced endogenous or existing flavonoid 3′-hydroxylase activity, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence encoding flavonoid 3′-hydroxylase or a derivative thereof, regenerating a transgenic plant from the cell and where necessary growing said

transgenic plant under conditions sufficient to permit the expression of the nucleic acid molecule.

29. A method according to claim 27 or 28 wherein the introduced nucleic acid molecule comprises a nucleotide sequence or complementary nucleotide sequence selected from:
(i) a nucleotide sequence encoding an amino acid sequence set forth in one of SEQ ID NO:2, 4, 6, 8, 10, 11, 12, 13, 15, 17, 19, 21, 23 or 25 or a complementary form thereof;
(ii) a nucleotide sequence set forth in one of SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22 or 24 or the coding region in SEQ ID NO:9 or a complementary form thereof;
(iii) a nucleotide sequence having at least about 60% similarity to (i) or (ii); and
(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to (i), (ii) and/or (iii).

30. A method according to claim 27 or 28 wherein the recipient plant is selected from petunia, carnation, chrysanthemum, rose, snapdragon, tobacco, cornflower, pelargonium, lisianthus, gerbera, apple, iris, lily, African violet and morning glory.

31. A method for producing a transgenic plant capable of modulating hydroxylation of flavonoid compounds, said method comprising stably transforming a cell or group of cells of a suitable plant with a nucleic acid molecule comprising a sequence of nucleotides encoding, or complementary to a sequence encoding, flavonoid 3′-hydroxylase or a derivative thereof, and regenerating a transgenic plant from said cell or cells.

32. A method according to claim 31 where the transformed nucleic acid molecule comprises a nucleotide sequence selected from:
(i) a nucleotide sequence encoding an amino acid sequence set forth in one of SEQ ID NO:2, 4, 6, 8, 10, 11, 12, 13, 15, 17, 19, 21, 23 or 25 or a complementary form thereof;
(ii) a nucleotide sequence set forth in one of SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22 or 24 or the coding region in SEQ ID NO:9 or a complementary form thereof;
(iii) a nucleotide sequence having at least about 60% similarity to (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to (i), (ii) and/or (iii).

33. A transgenic plant having tissue exhibiting altered colour, said transgenic plant comprising a nucleic acid molecule comprising a sequence of nucleotides selected from:
(i) a nucleotide sequence encoding an amino acid sequence set forth in one of SEQ ID NO:2, 4, 6, 8, 10, 11, 12, 13, 15, 17, 19, 21, 23 or 25 or a complementary form thereof;
(ii) a nucleotide sequence set forth in one of SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22 or 24 or the coding region in SEQ ID NO:9 or a complementary form thereof;
(iii) a nucleotide sequence having at least about 60% similarity to (i) or (ii); and
(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to (i), (ii) and/or (iii).

34. A cut flower from a transgenic plant according to claim 33.

35. A seed from a transgenic plant according to claim 33.

36. A fruit from a transgenic plant according to claim 33.

37. A leaf from a transgenic plant according to claim 33.

38. Use of a nucleic acid molecule comprising a sequence of nucleotides encoding a flavonoid 3′-hydroxylase in the manufacture of a genetic construct capable of modulating hydroxylation of flavonoid compounds in a plant or cells of a plant.

39. Use according to claim 38 wherein the nucleotide sequence is selected from:
(i) a nucleotide sequence encoding an amino acid sequence set forth in one of SEQ ID NO:2, 4, 6, 8, 10, 11, 12, 13, 15, 17, 19, 21, 23 or 25 or a complementary form thereof;
(ii) a nucleotide sequence set forth in one of SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22 or 24 or the coding region in SEQ ID NO:9 or a complementary form thereof;

(iii) a nucleotide sequence having at least about 60% similarity to (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to (i), (ii) and/or (iii).

Figure 1a

Figure 1b

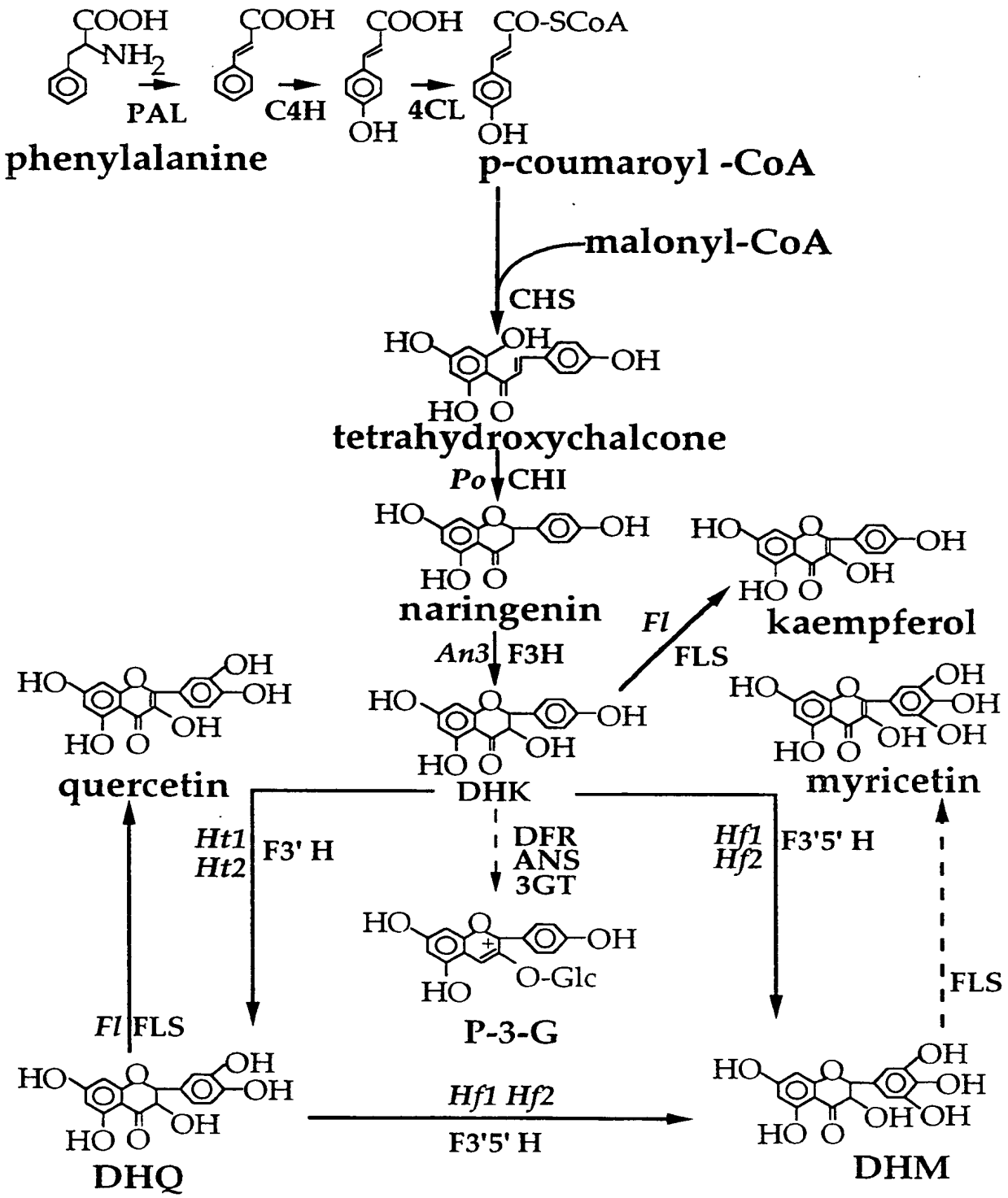

COOH
NH2
PAL
COOH
C4H
COOH
OH
4CL
CO-SCoA
OH
phenylalanine
p-coumaroyl -CoA
malonyl-CoA
CHS
HO
OH
OH
HO O
tetrahydroxychalcone
Po CHI
naringenin
kaempferol
Fl
FLS
An3 F3H
quercetin
DHK
myricetin
Ht1
Ht2
F3' H
DFR
ANS
3GT
Hf1
Hf2
F3'5' H
O-Glc
P-3-G
FLS
Fl FLS
Hf1 Hf2
F3'5' H
DHQ
DHM

Figure 1a

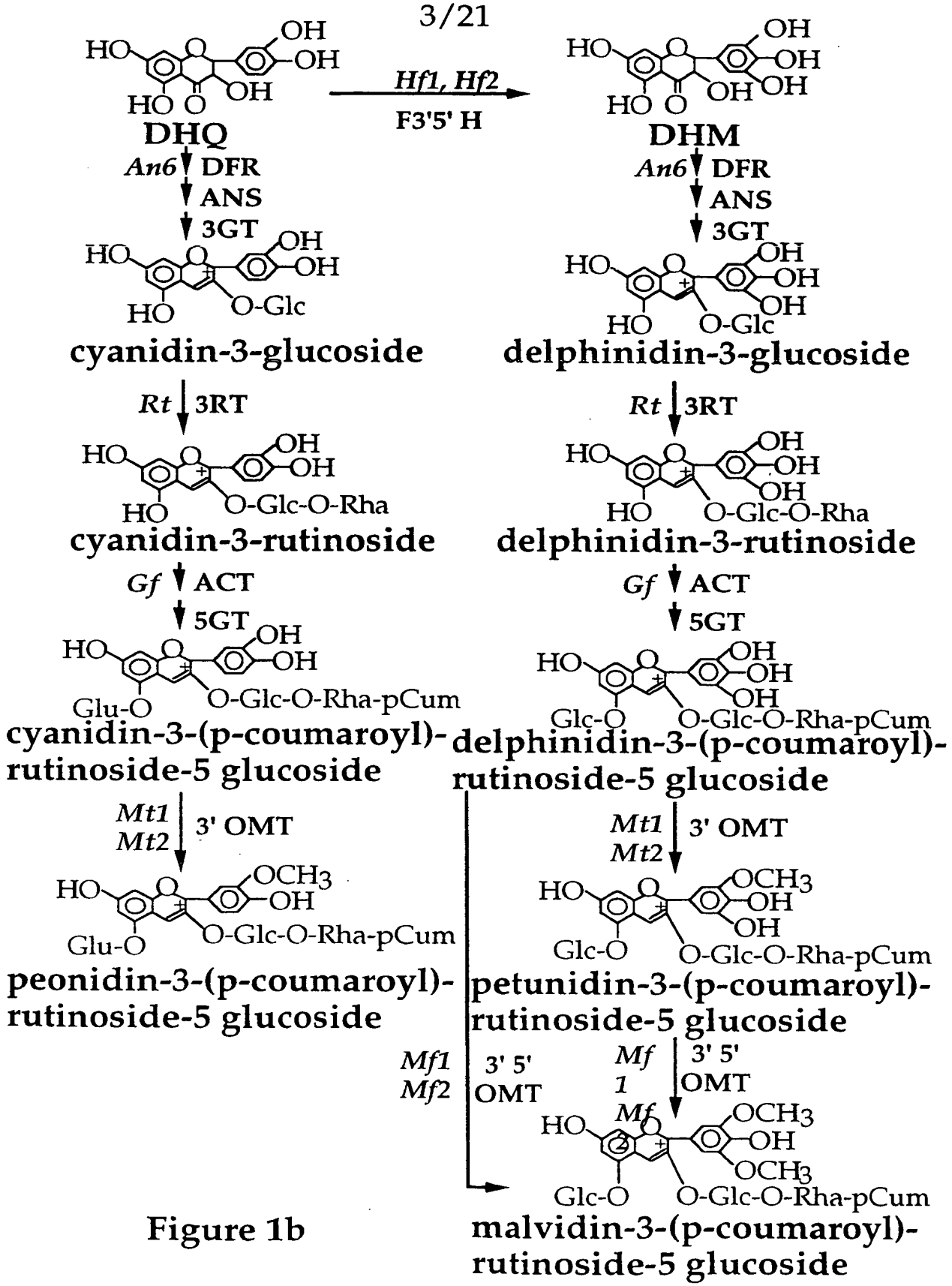

DHQ
Hf1, Hf2
F3'5' H
DHM
An6 DFR
ANS
3GT
cyanidin-3-glucoside
delphinidin-3-glucoside
Rt 3RT
cyanidin-3-rutinoside
delphinidin-3-rutinoside
Gf ACT
5GT
cyanidin-3-(p-coumaroyl)-rutinoside-5 glucoside
delphinidin-3-(p-coumaroyl)-rutinoside-5 glucoside
Mt1 Mt2 3' OMT
peonidin-3-(p-coumaroyl)-rutinoside-5 glucoside
petunidin-3-(p-coumaroyl)-rutinoside-5 glucoside
Mf1 Mf2 3' 5' OMT
malvidin-3-(p-coumaroyl)-rutinoside-5 glucoside

Figure 1b

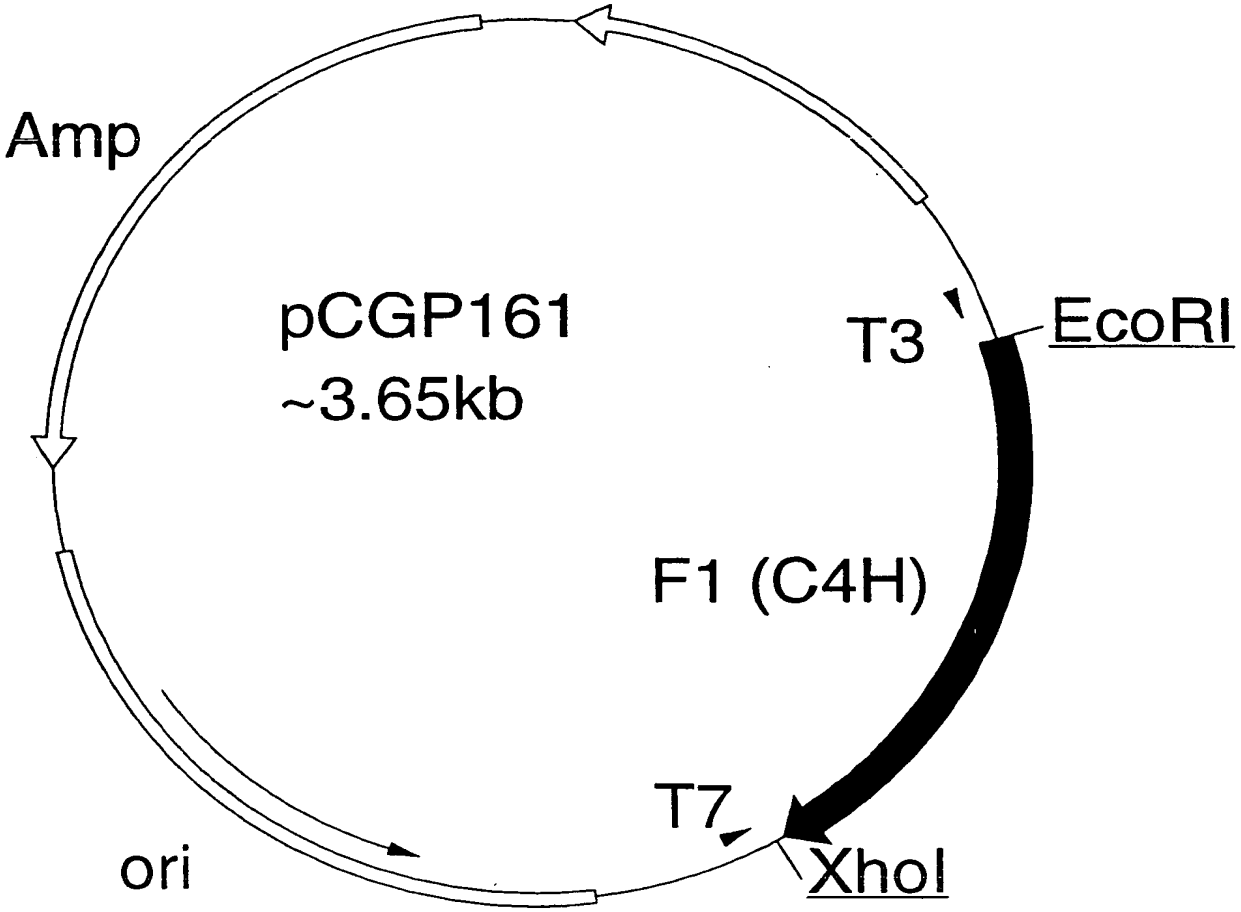
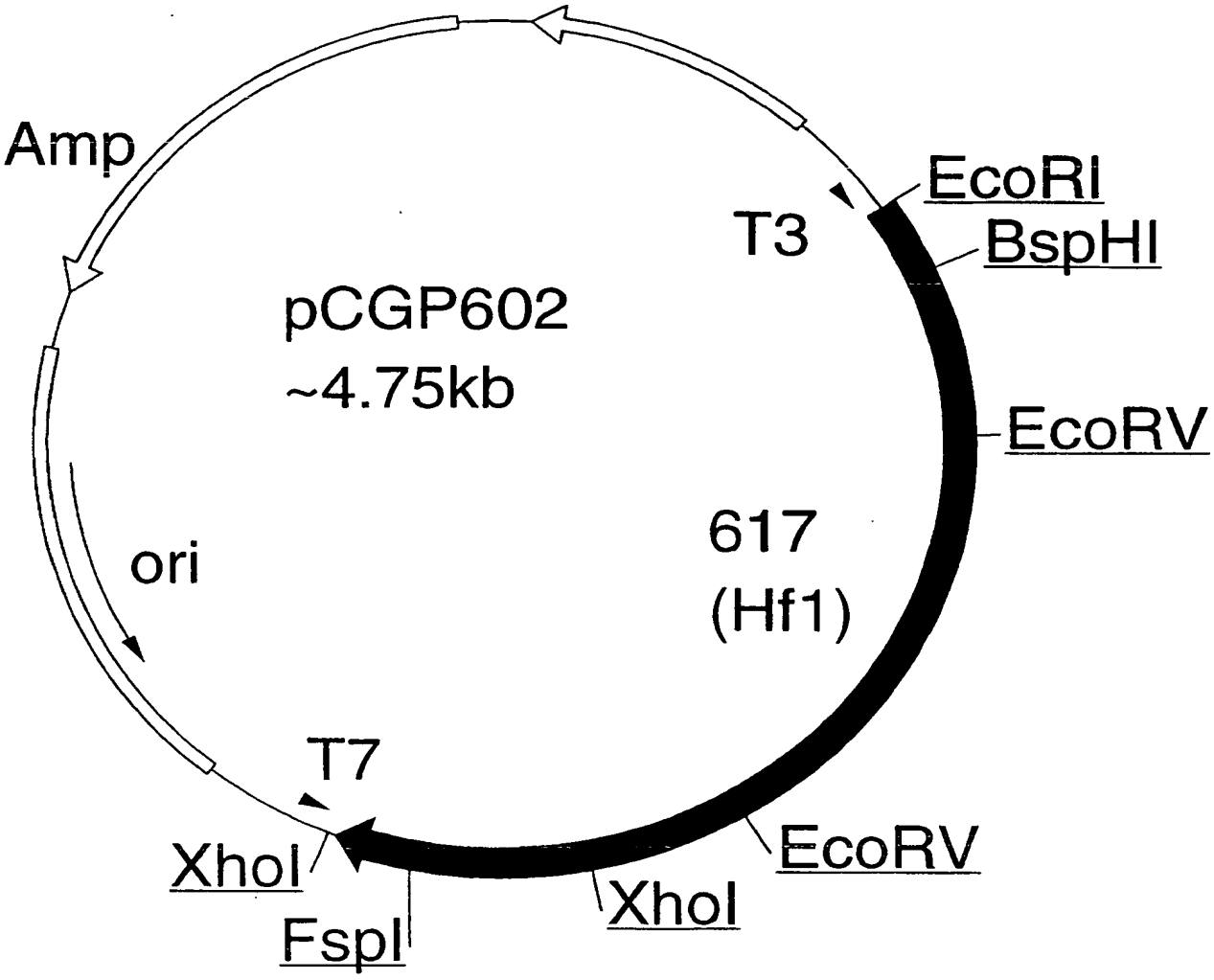


Figure 2


Amp
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T3
BspHI
pCGP602
~4.75kb
EcoRV
617
(Hf1)
ori
T7
EcoRV
XhoI
XhoI
FspI

Figure 3

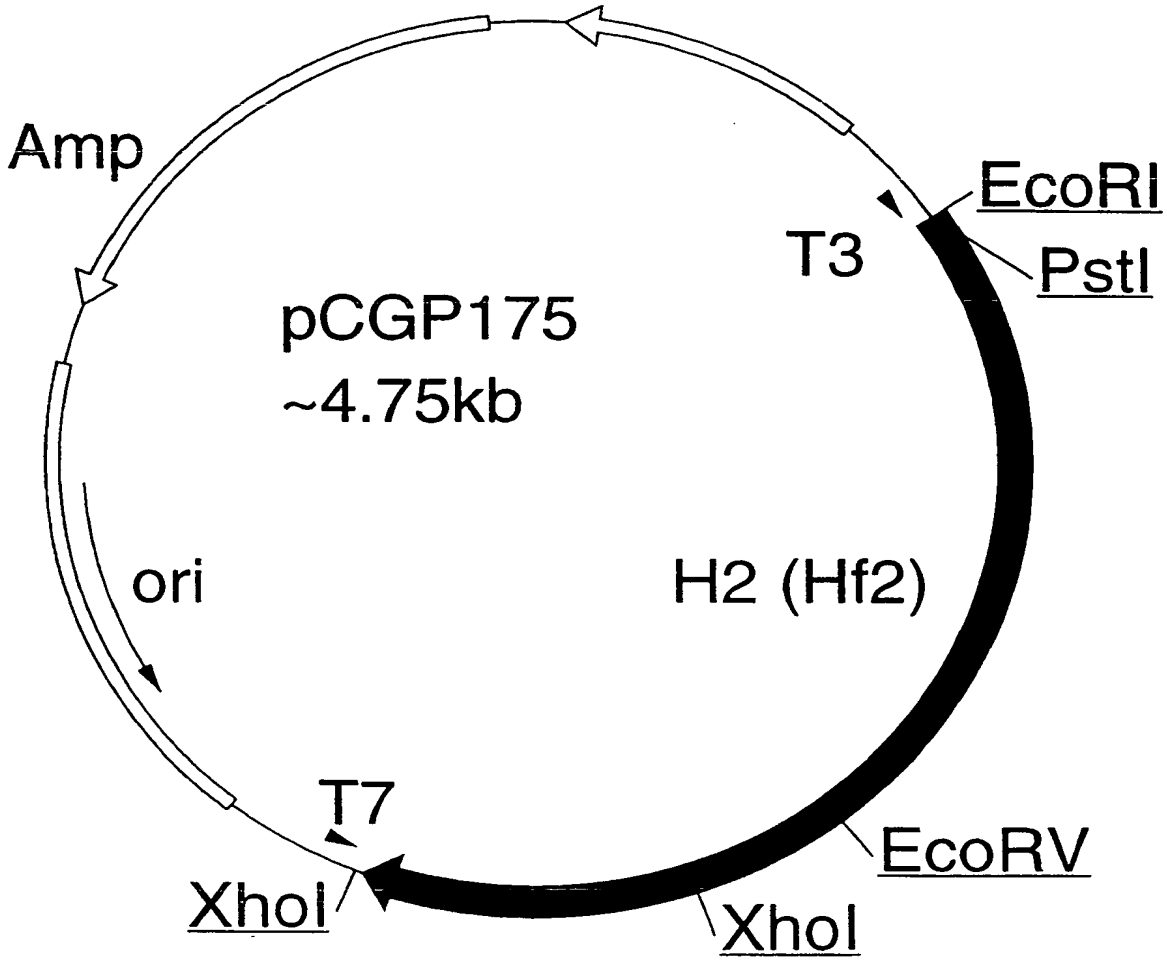


Figure 4

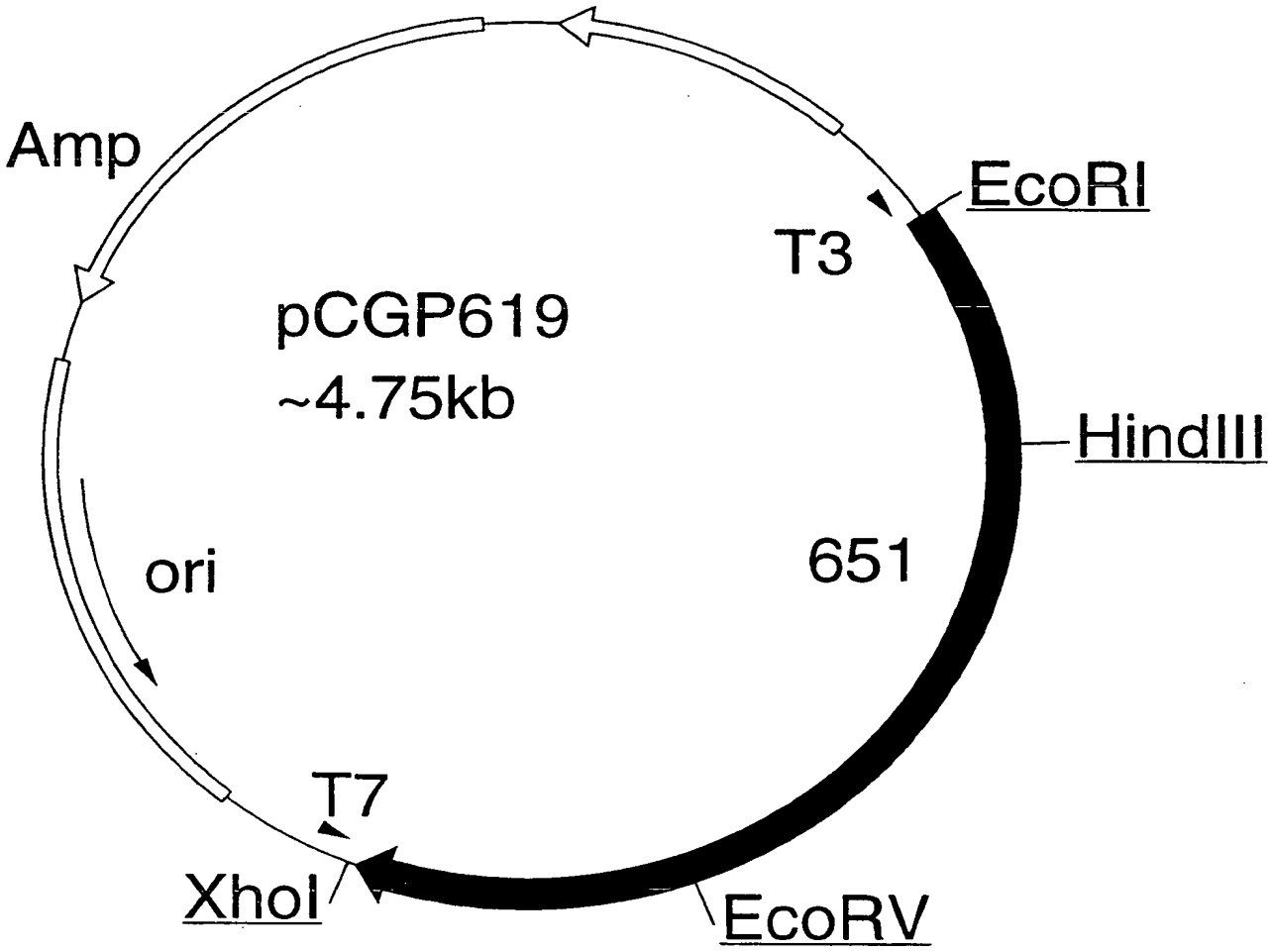


Figure 5

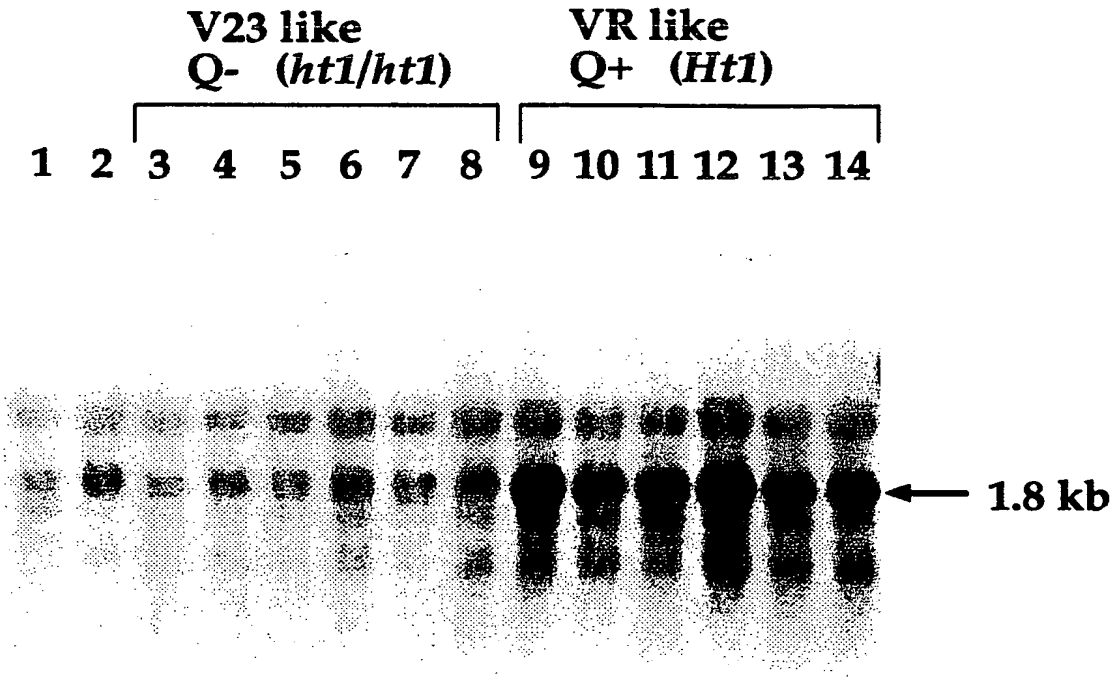
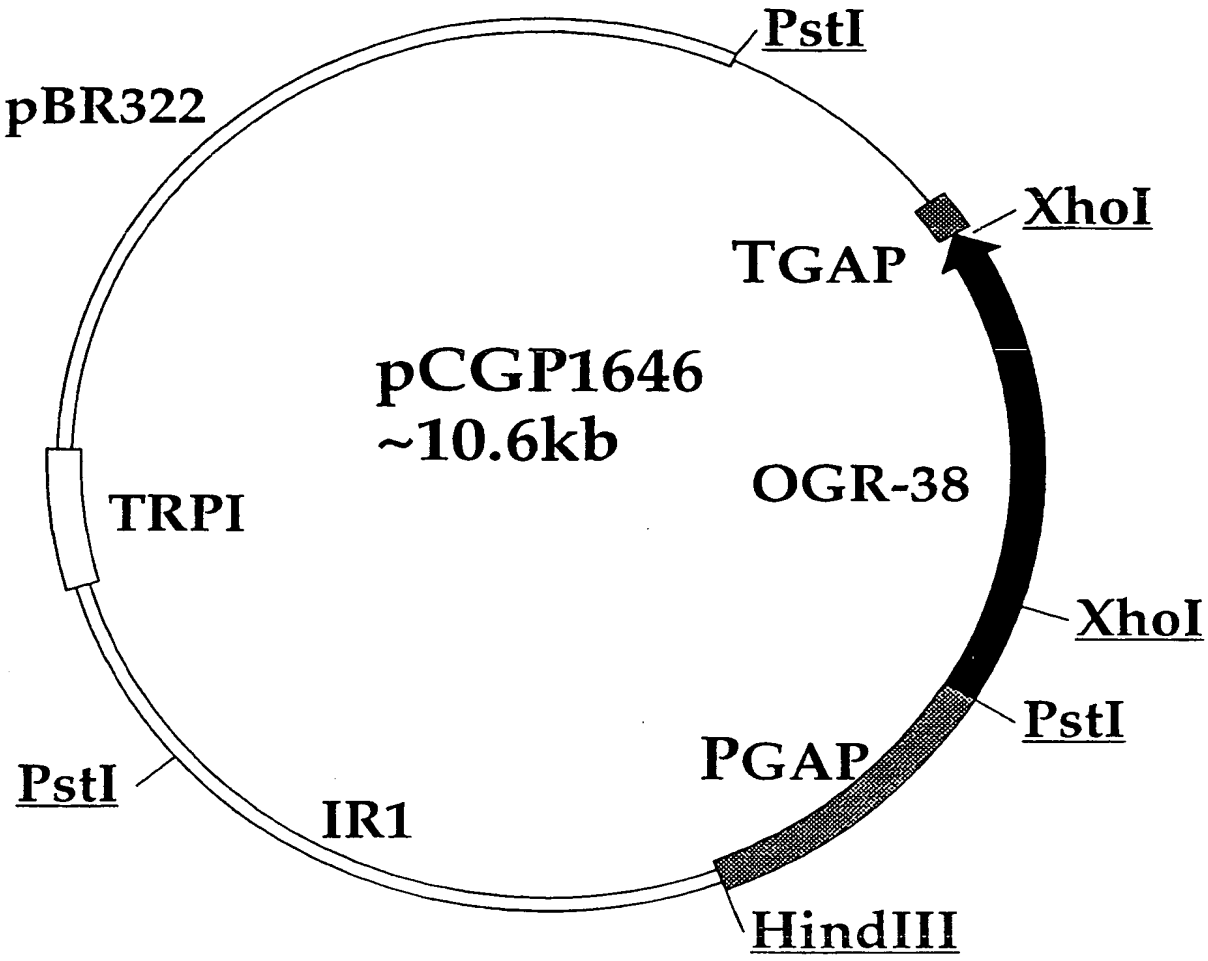


Figure 6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pCGP1646
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OGR-38
TRPI
XhoI
PstI
PGAP
PstI
IR1
HindIII

Figure 7

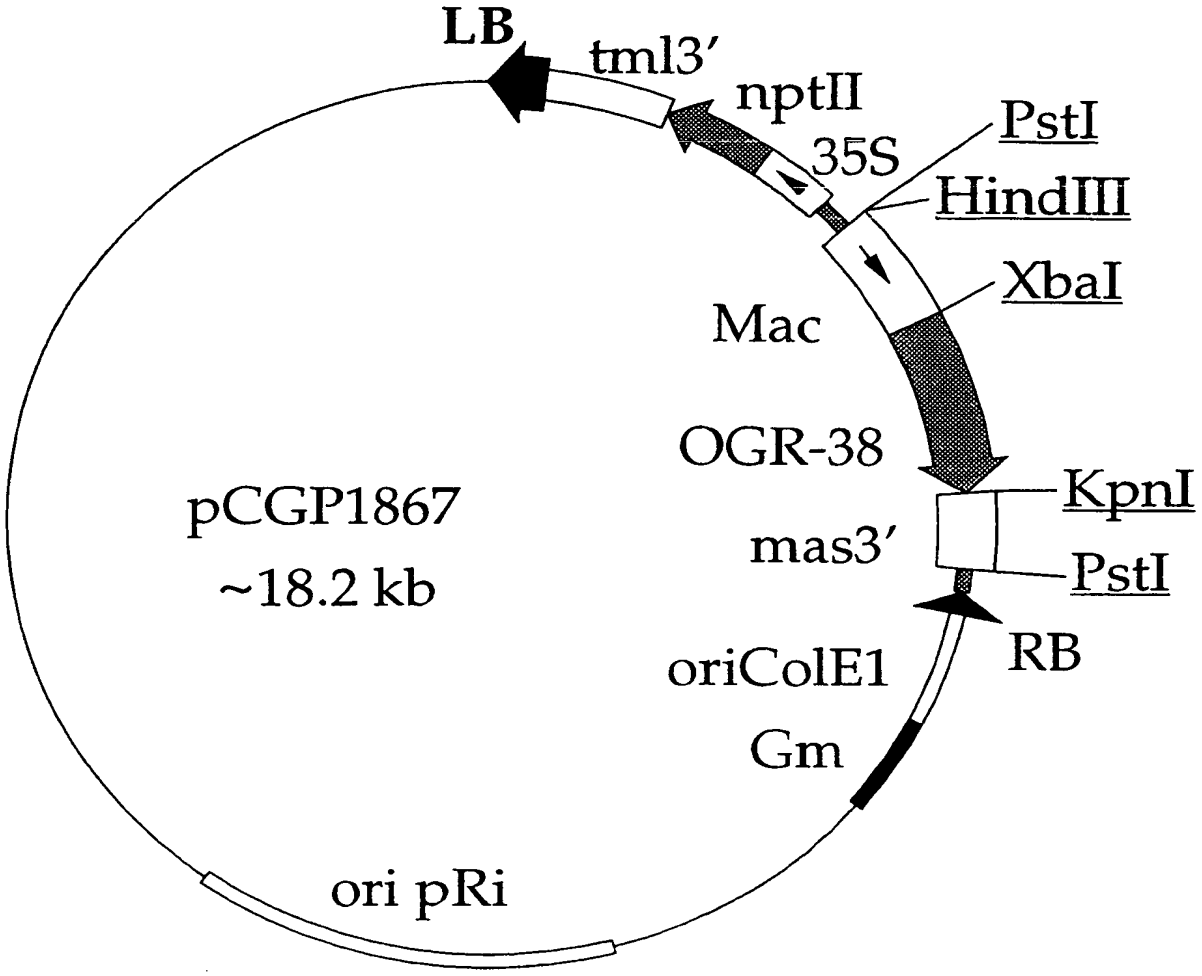
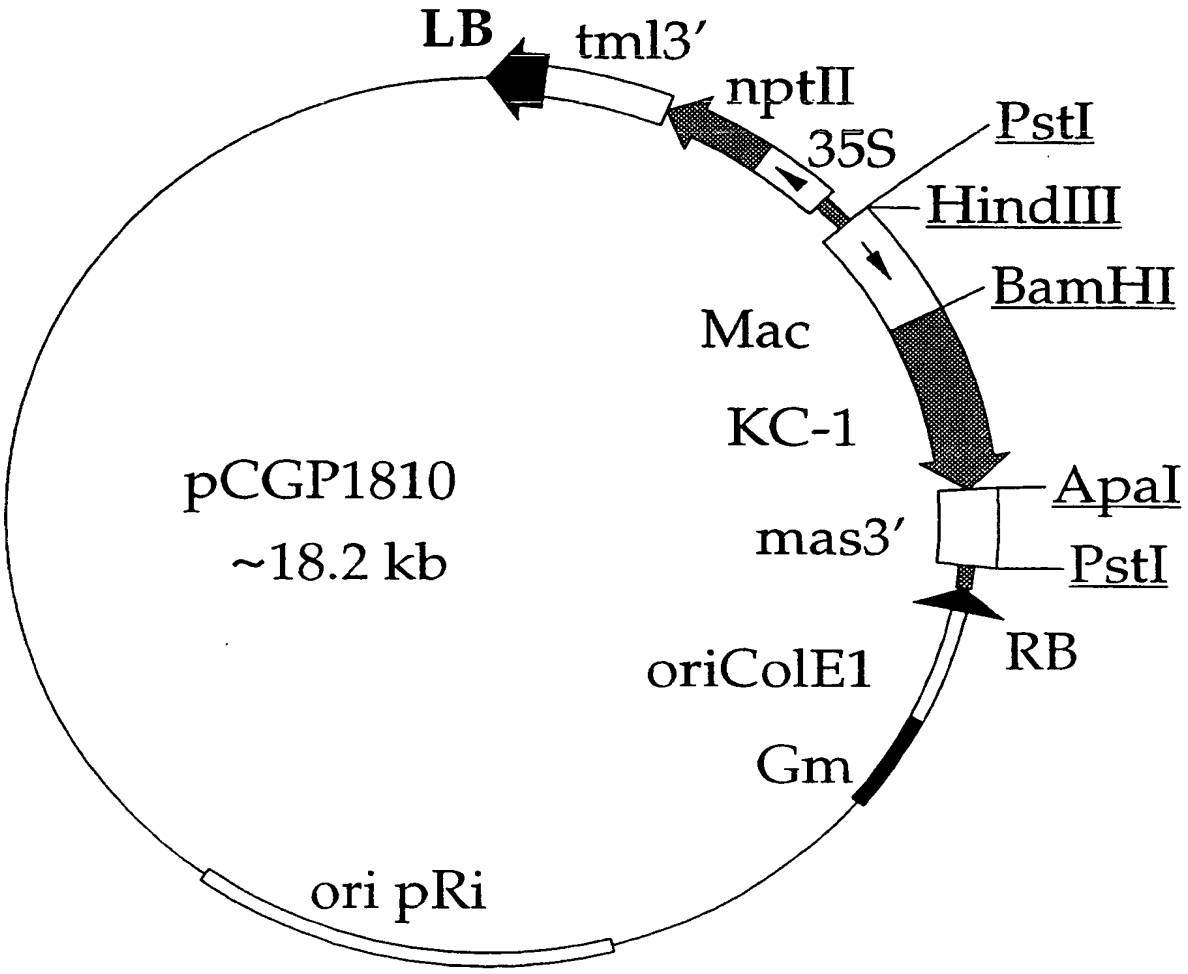

LB
tml3′
nptII
35S
PstI
HindIII
XbaI
Mac
OGR-38
KpnI
mas3′
PstI
RB
oriColE1
Gm
ori pRi
pCGP1867
~18.2 kb

Figure 8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35S
PstI
HindIII
BamHI
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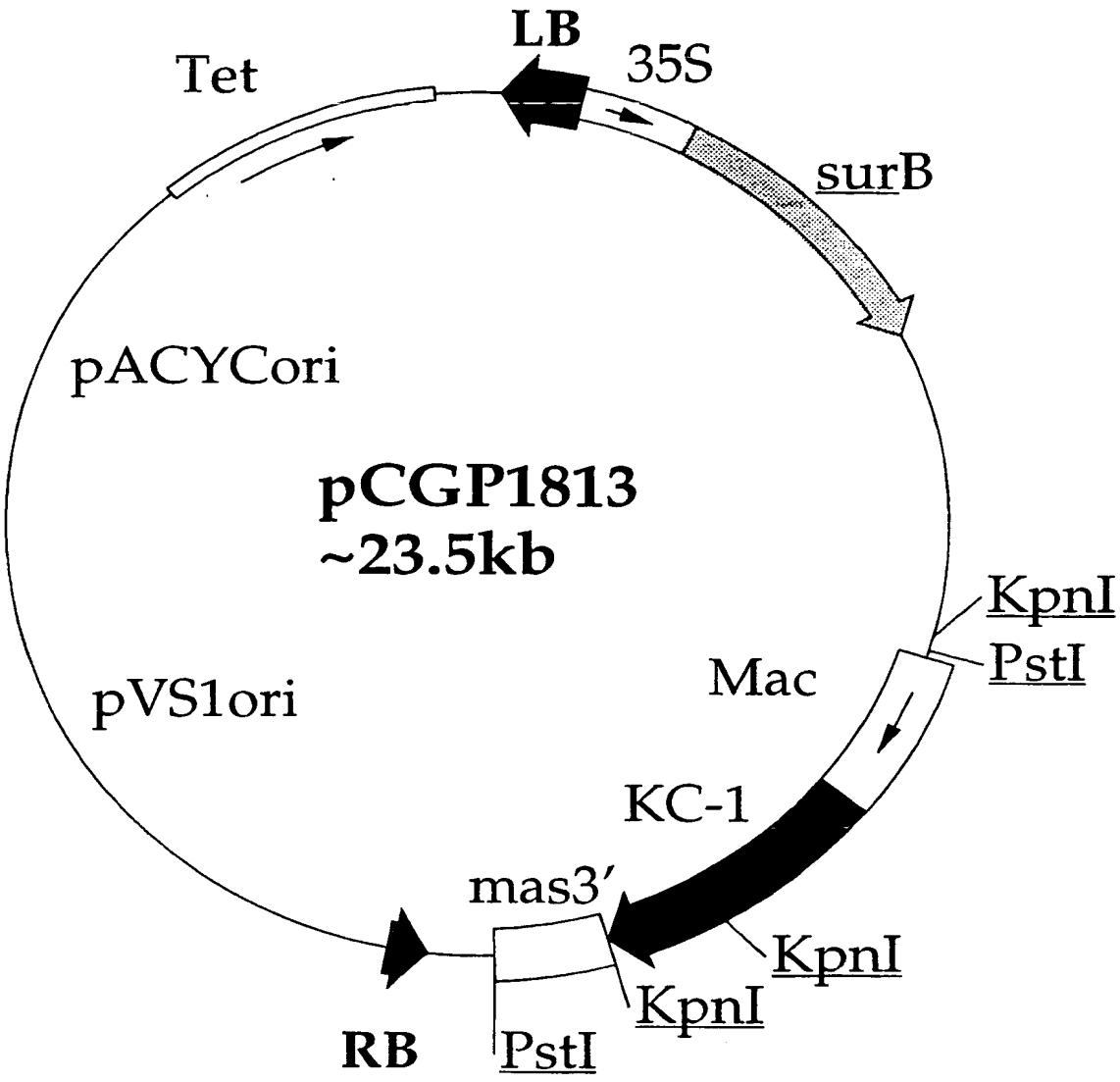
KC-1
pCGP1810
~18.2 kb
ApaI
mas3′
PstI
RB
oriColE1
Gm
ori pRi

Figure 9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surB
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pCGP1813
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PstI
Mac
pVS1ori
KC-1
mas3′
KpnI
KpnI
RB
PstI

Figure 10

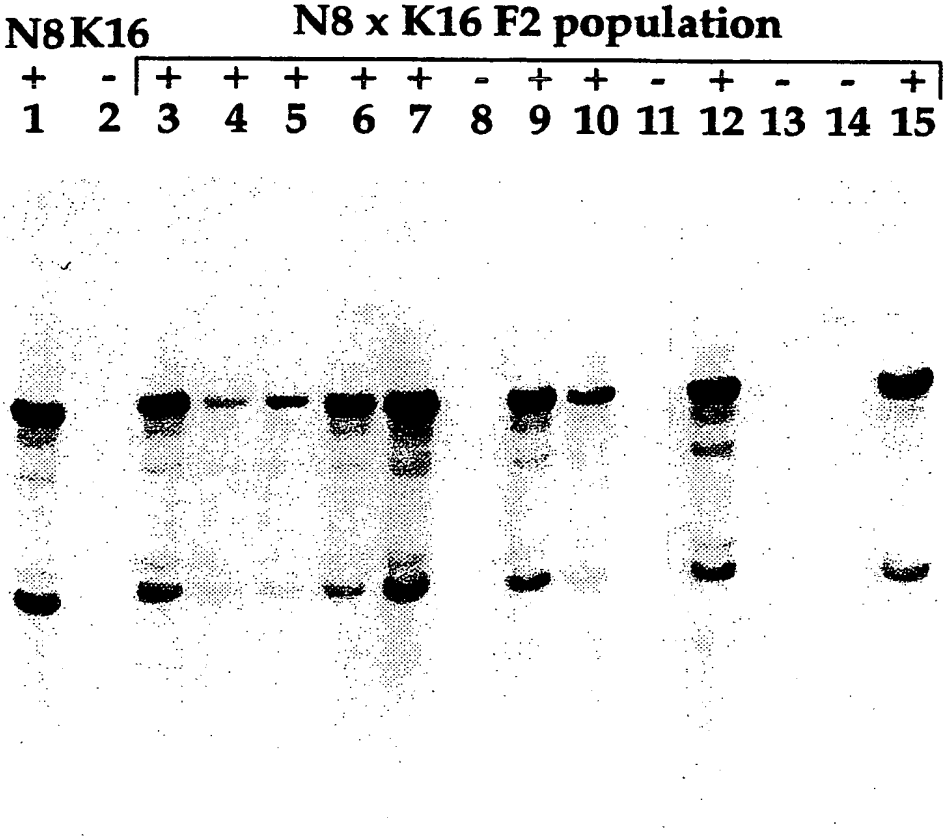


Figure 11

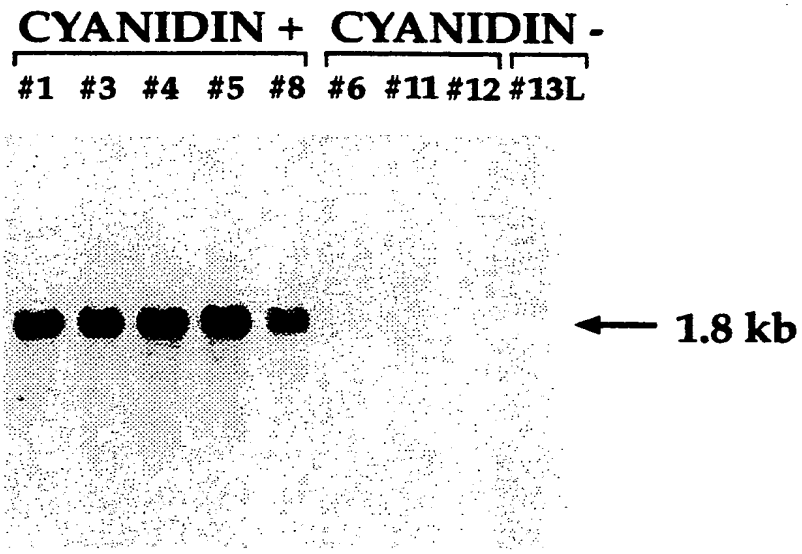
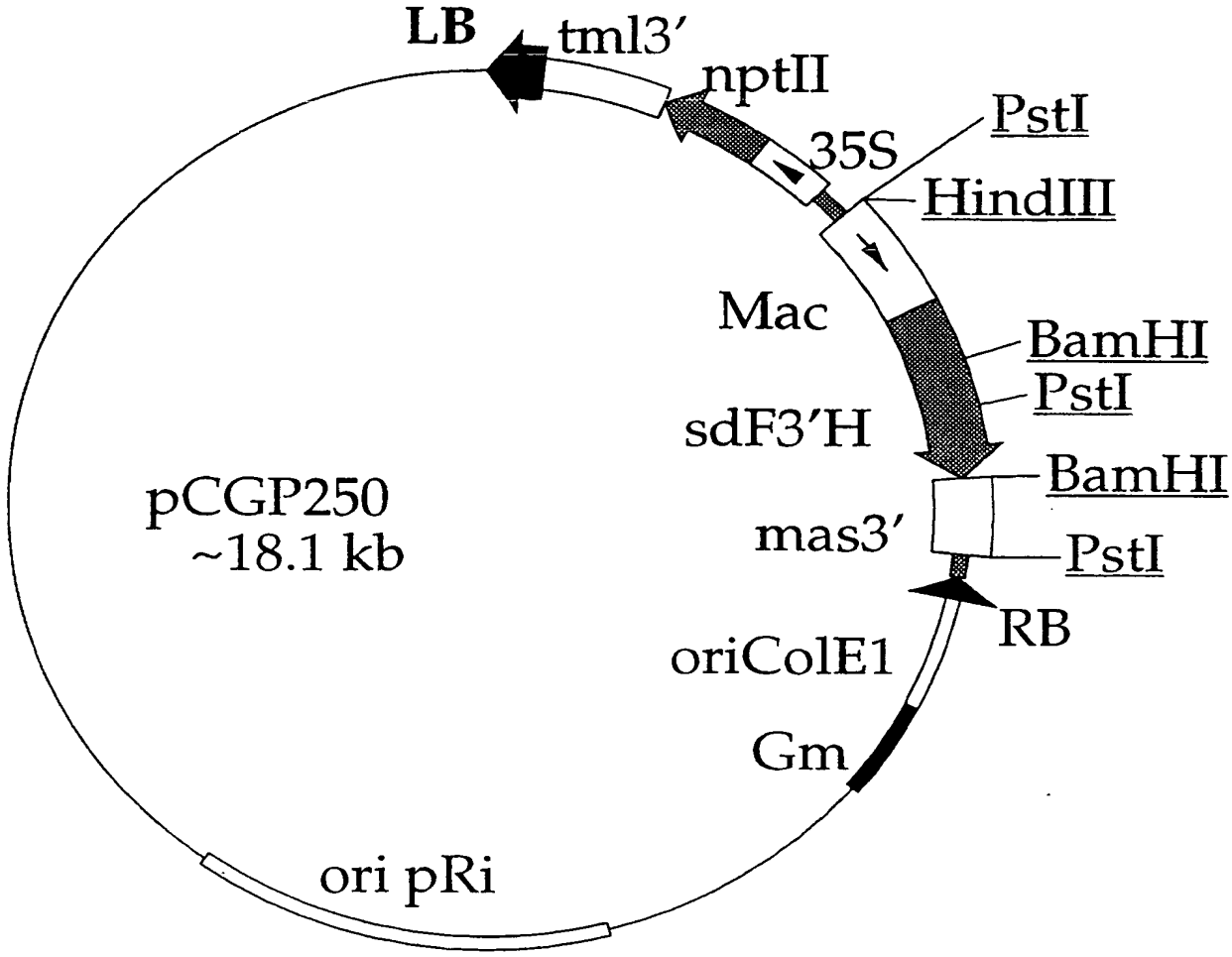


Figure 12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PstI
35S
HindIII
Mac
BamHI
PstI
sdF3′H
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pCGP250
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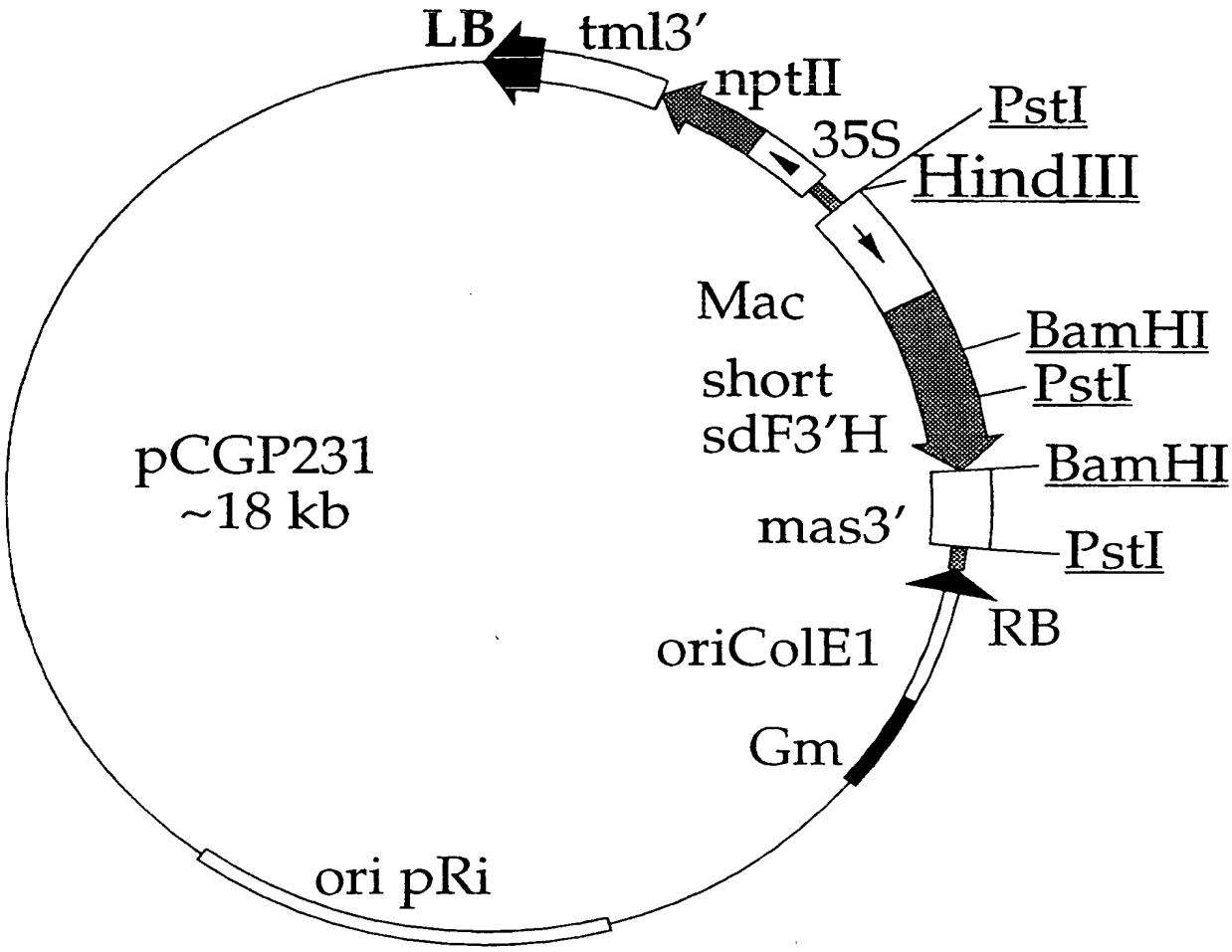
mas3′
PstI
RB
oriColE1
Gm
ori pRi

Figure 13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tml3′
nptII
35S
PstI
HindIII
Mac
BamHI
short
PstI
sdF3′H
pCGP231
~18 kb
BamHI
mas3′
PstI
RB
oriColE1
Gm
ori pRi

Figure 14

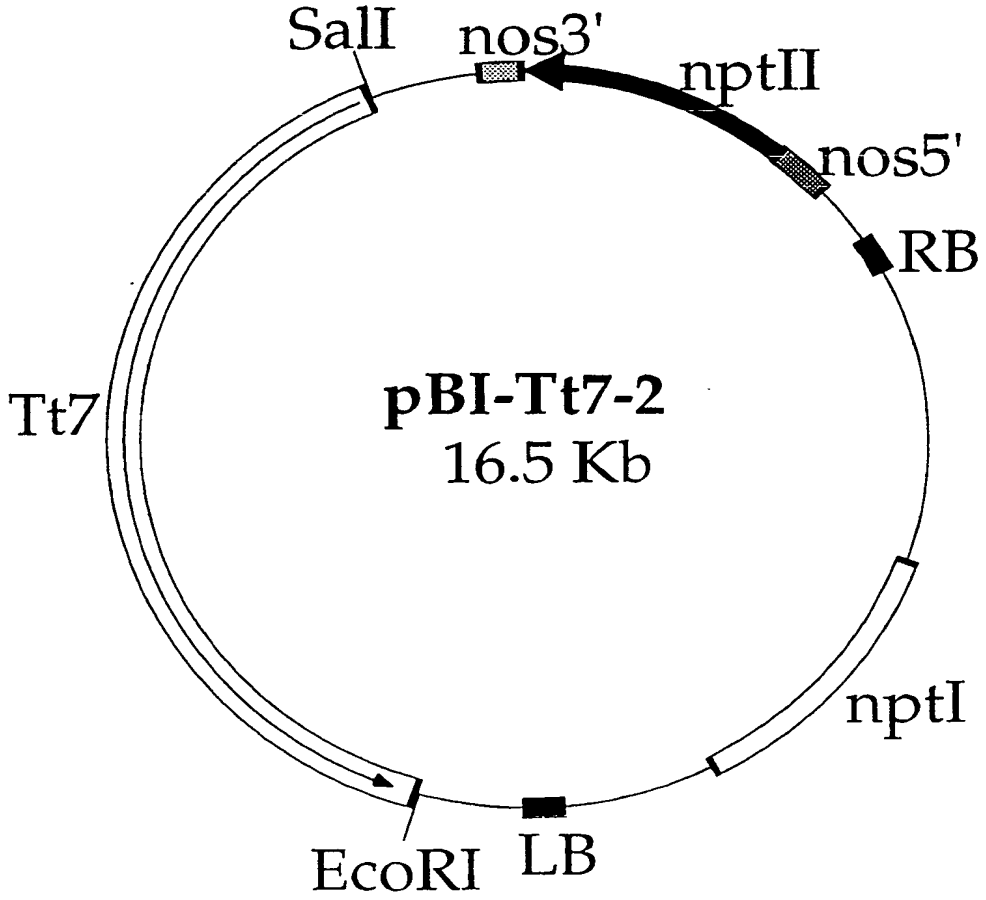
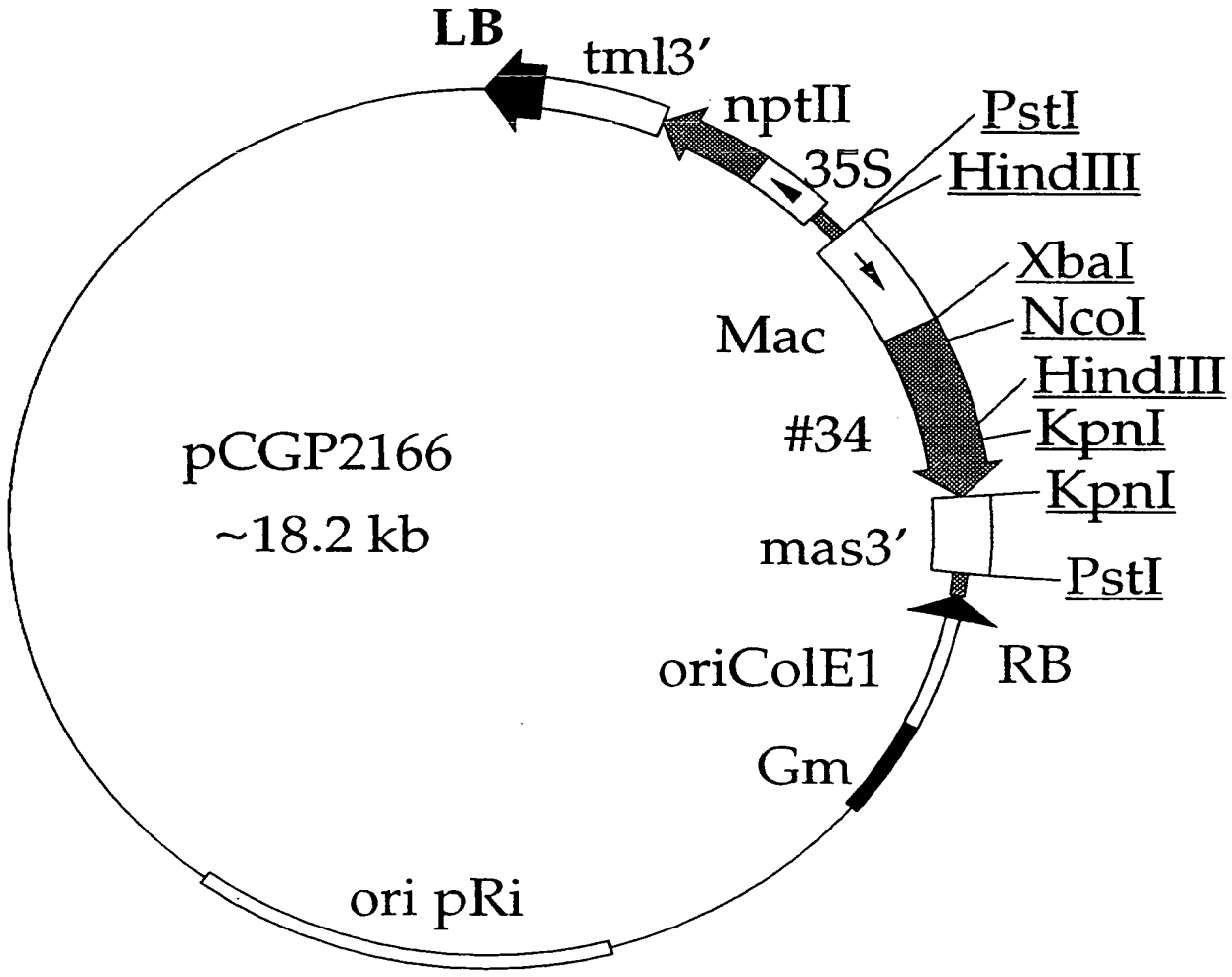


Figure 15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HindIII
XbaI
NcoI
Mac
HindIII
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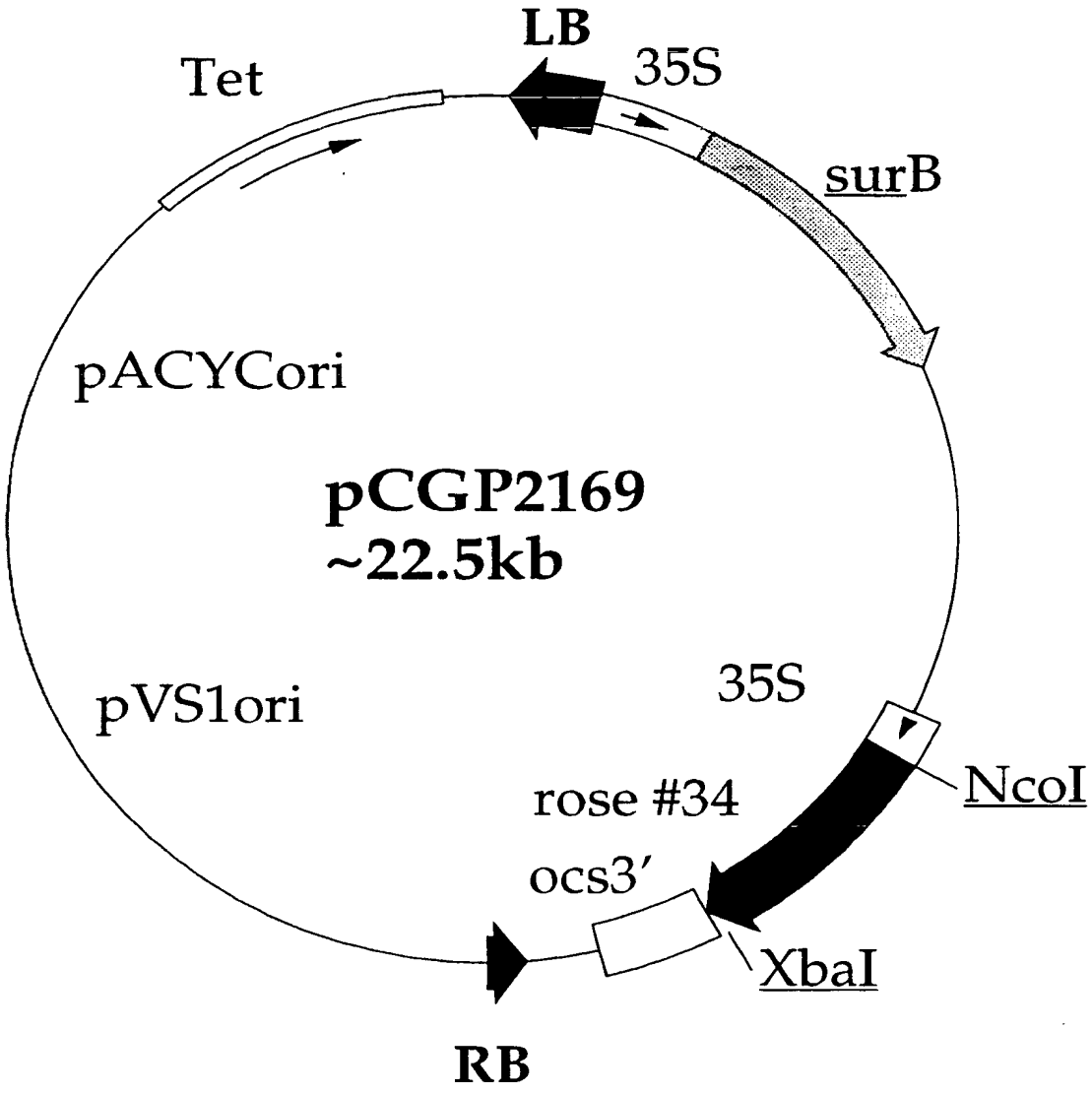
KpnI
mas3′
PstI
oriColE1
RB
Gm
ori pRi

Figure 16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Tet
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pVS1ori
35S
NcoI
rose #34
ocs3'
XbaI
RB

Figure 17

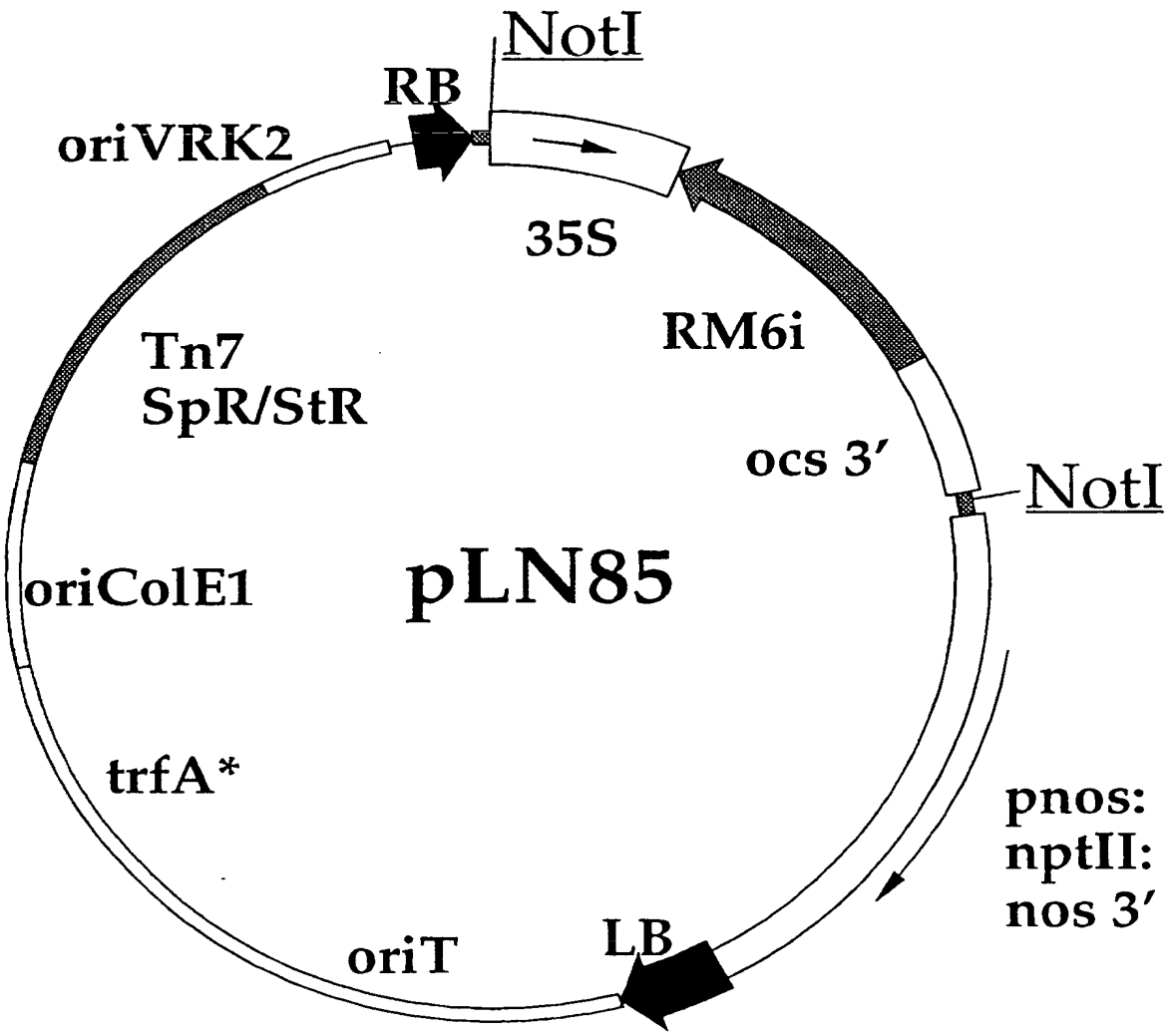
NotI
RB
oriVRK2
35S
RM6i
Tn7
SpR/StR
ocs 3′
NotI
oriColE1
pLN85
trfA*
pnos:
nptII:
nos 3′
oriT
LB

Figure 18

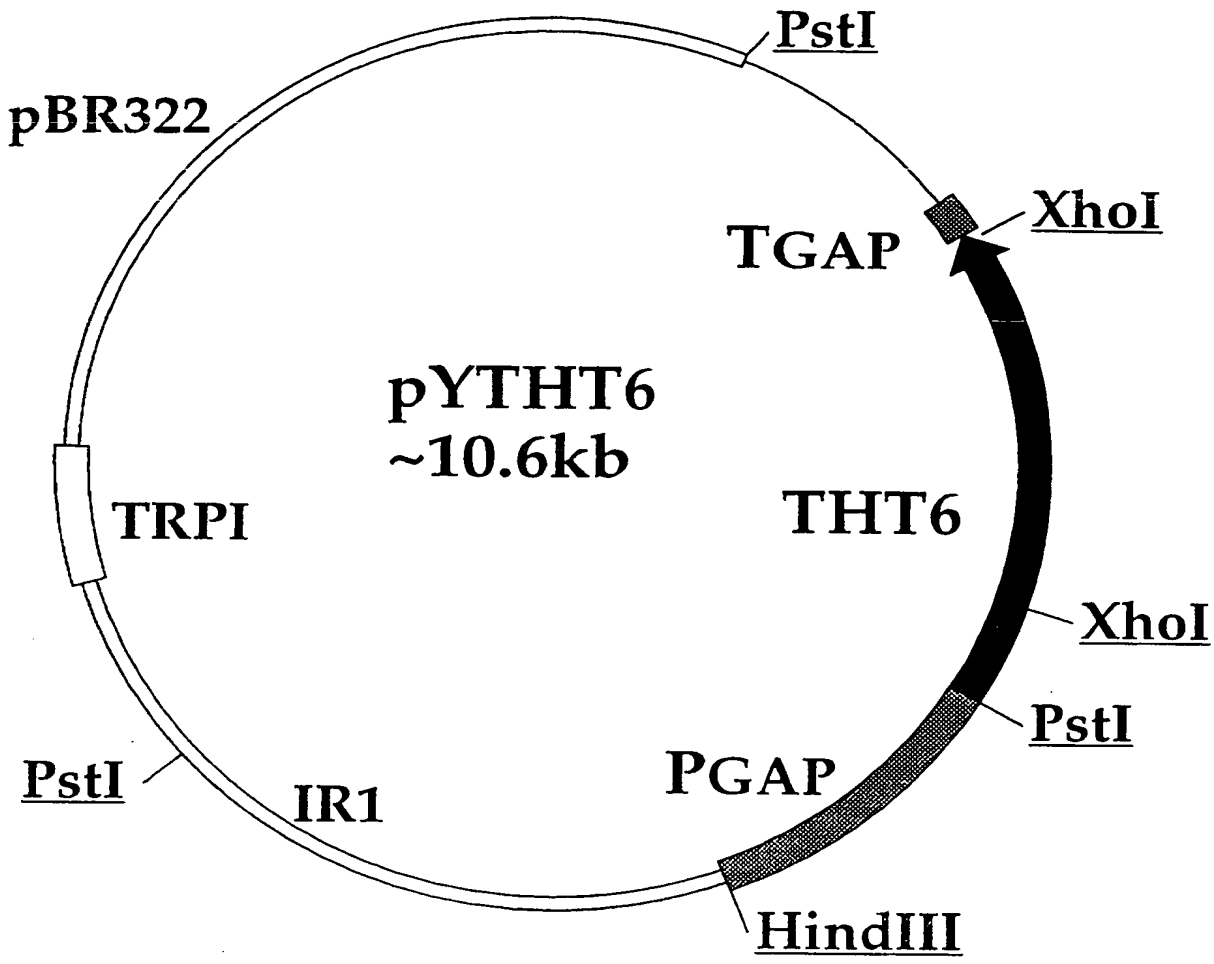
PstI
pBR322
XhoI
TGAP
pYTHT6
~10.6kb
TRPI
THT6
XhoI
PstI
PstI
PGAP
IR1
HindIII

Figure 19

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00124

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/29, 15/63
A01H 5/00, 5/02

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See electronic databases below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields scarched
See electronic databases below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO, USPM - ss1: flavonoid(3n)monooxygenase# or flavonoid(3n)hydroxylase# or F3()H; ss2: A01H/IC or C12N-009/IC or C12N-015/IC; ss1 and ss2
CHEMICAL ABSTRACTS - flavonoid 3' hydroxylase (clones pcgp772, pcgp602, pcgp619, pcgp635, pcgp854, pcgp773)/cn; flavonoid 3' hydroxylase/cn, flavonoid 3' monooxygenase
GENEBANK, EMBL, SWISS-PROT - Sequence searches

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU A 37413/93 (INTERNATIONAL FLOWER DEVELOPMENTS PTY. LTD.) 14 OCTOBER 1993 C12N 15/53, 15/11, 9/02, C12Q 1/66, A01H 5/00	27,28,30,31,38
A	PHYTOCHEMISTRY, VOL. 35 PAGES 145-150, 1994, SCHWINN KE et al. "Floral flavonoids and the potential for pelargonidin biosynthesis in commercial chrysanthemum cultivars"	1 - 38

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 1997	22 APR 1997
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer **KAREN AYERS** Telephone No.: (06) 283 2082

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 97/00124

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
AU, A,	37413/93	WO, A,	93/20206
		EP, A,	640136
		NZ, A,	249808

END OF ANNEX